DISSERTATION ON

A COMPARATIVE ANALYSIS OF HIGH-SENSITIVITY C-REACTIVE PROTEIN (hsCRP) AND FIBRINOGEN LEVEL IN TYPE 2 DIABETICS AND MATCHED CONTROLS

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CERTIFICATE

This is to certify that this dissertation entitled "**A COMPARATIVE ANALYSIS OF HIGH SENSITIVITY C-REACTIVE PROTEIN (hsCRP) AND FIBRINOGEN LEVEL IN TYPE 2 DIABETICS AND MATCHED CONTROLS**" submitted by **Dr. TEFFY JOSE,** appearing for Part II M.D. Branch I General Medicine Degree examination in April 2012 is a bonafide record of work done by her under my direct guidance and supervision in partial fulfillment of regulations of the Tamil Nadu Dr. M.G.R. Medical University, Chennai. I forward this to the Tamil Nadu Dr.M.G.R. Medical University, Chennai, Tamil Nadu, India.

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DECLARATION

I solemnly declare that the dissertation entitled "**A COMPARATIVE ANALYSIS OF HIGH SENSITIVITY C-REACTIVE PROTEIN (hsCRP) AND FIBRINOGEN LEVEL IN TYPE 2 DIABETICS AND MATCHED CONTROLS**" is done by me at the Government Stanley Medical College and Hospital, Chennai during 2011 under the guidance and supervision of Dr.K.Madhavan, M.D.

This dissertation is submitted to The Tamilnadu Dr. M.G.R Medical University, towards partial fulfillment of regulation for the award of M.D. DEGREE IN GENERAL MEDICINE (BRANCH–I).

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CONTENTS

INTRODUCTION

Diabetes mellitus (DM) is a heterogenous group of metabolic disorders recognized from ancient era which is characterized by chronic hyperglycemia. The prevalence of diabetes especially type 2 DM is rapidly increasing worldwide over the past two decades. In 2010, there were 285 million individuals worldwide with type 2 diabetes[1]. India had 41 million diabetics in 2006, according to the International Diabetic Federation (IDF) and it is expected to increase to 70 million by the year 2025[2]. Diabetes was responsible for almost four million deaths in 2010 (6.8% of deaths) and is the fifth leading cause of mortality worldwide[3]. Cardiovascular complications especially coronary heart disease are the most common cause of morbidity and mortality in diabetics[4].

Diabetes is an independent risk factor for cardiovascular disease[4] and is designated as a "CHD risk equivalent" by the American Heart Association[5]. The relative risk of cardiovascular disease is two to four fold higher in people with diabetes[6].The probability for a diabetic person to get a first myocardial infarction is equal to that of a non diabetic person getting it for the second time.[7]. The increased prevalence of CVD in diabetes has been attributed in large part to the acceleration of coronary atherosclerosis, which occurs at an earlier age and advances more rapidly to clinical cardiovascular events in individuals with diabetes, with

contribution from conventional risk factors for cardiovascular disease such as hypertension, dyslipidemia and smoking[8]. A graded relationship also exists between glycemic control (estimated by HbA1C level) and cardiovascular risk [9].

Diabetes is now considered to be a proinflammatory[10] and procoagulant[11] state and low grade chronic inflammation ('microinflammation')[10] is a key factor in the genesis and rupture of atheromatous plaque. Hyperglycemia, oxidative stress, inflammation and dysregulation of hemostasis contribute to the increased risk of diabetic vasculopathies. Hence, the diabetes state per se confers an increased propensity to accelerated atherogenesis, which is compounded by other conventional cardiovascular risk factors[12].

C reactive protein, measured as high sensitivity C reactive protein (hsCRP) and fibrinogen are acute phase reactants synthesized in the liver in response to proinflammatory cytokines. These markers of inflammation are found to be elevated in individuals with type 2 DM and are emerging as novel risk factors to assess cardiovascular risk. They also have positive association with obesity, dyslipidemia, hypertension and poor glycemic control[13].

In our study, we analyzed the levels of hsCRP and fibrinogen in individuals with type 2 DM and matched controls as a predictor of cardiovascular risk and its relationship to glycemic control.

AIMS AND OBJECTIVES

1. To assess and compare the high sensitivity C-reactive protein (hsCRP) level in patients with type 2 diabetes without clinically demonstrable vascular complications and that in matched controls.

2. To assess and compare the plasma fibrinogen level in patients with type 2 diabetes without clinically demonstrable vascular complications and that in matched controls.

3. To determine whether there is any correlation between high sensitivity C-reactive protein (hsCRP) level and the degree of glycemic control in patients with type 2 diabetes.

4. To determine whether there is any correlation between plasma fibrinogen level and the degree of glycemic control in patients with type 2 diabetes.

REVIEW OF LITERATURE

For 2000 years, diabetes mellitus was recognized as a devastating and deadly disease.

DEFINITION

Diabetes is a heterogenous group of metabolic disorders characterized by hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both[14].

THE HISTORY OF DIABETES

Earliest known record of diabetes was mentioned in 1552 BC, by Egyptian physician Hesy-Ra of the 3rd Dynasty on the Ebers Papyrus[15-17]. The term “diabetes” was coined by a Greek physician, Aretaeus of Cappadocia in the 1st century AD from the Greek word for “siphon”[18,19].

The word “mellitus” was added by Thomas Willis in the year 1675, from the Latin word meaning honey, a reference to the sweet taste of urine[16,19,20]. In 1776, Matthew Dobson confirmed that the sweet taste was because of an excess kind of sugar in the urine and blood of people with diabetes[16].

In 1889, the role of pancreas in the pathogenesis of diabetes was first described by Joseph von Mering and Oskar Minkowsky of the University of Strasbourg, France[16,21]. In 1909, Jean De Meyer called the glucose lowering hormone produced by islet tissue as 'insulin'[16].

In 1921, the discovery and extraction of insulin at the University of Toronto by a collaborate effort involving Sir Frederick G Banting, Charles H Best, James B Collip and J J R MacLeod revolutionized the history of diabetes mellitus. In 1922, Leonard Thompson, aged 14, became the first person to receive insulin injection to treat diabetes[16,22]. For this, Banting and MacLeod received the Nobel Prize in Physiology or Medicine in the year 1923. WHO, from the year 2007 onwards, celebrates World Diabetes Day on November 14, on the birthday of Sir Frederick G Banting, in his honour.

Eli Lilly began the commercial production of insulin (Isletin insulin) in 1923. In 1936, Sir Harold Himsworth of the University College Hospital in London proposed that diabetes falls into two types based on 'insulin insensitivity'. In 1940s link was made between diabetes and long term complications of eye and kidney diseases. In 1942, sulphonyl urea, the first oral hypoglycemic agent was discovered by M J Janbon[16,23].

In 1976, the glycosylated hemoglobin (A1C) test to monitor glycemic control was introduced[24,25]. In 1978, first recombinant DNA insulin was produced[16,24]. In 1983, first biosynthetic insulin was introduced. “Reflolux”, later known as “ Accu-Chek” which allows blood glucose self monitoring was introduced in the same year. In 2001, FDA approved Glucowatch Biographer, the first noninvasive glucose monitor for adults. Later Oral-Lyn, an oral spray formulation of human insulin (2005) and Inhaled insulin (Exubera) (2006)[26] was introduced.

EPIDEMIOLOGY OF DIABETES

WORLDWIDE

There is a dramatic increase in the prevalence of diabetes mellitus especially type 2 DM, worldwide over the past two decades. It has increased from 30 million cases in 1985 to 285 million in 2010 and is projected to increase to the level of 438 million by the year 2030, according to the International Diabetic Federation[1]. The greatest number of individuals with diabetes will be aged 45-64 years in 2030, according to worldwide estimates. The increase in the prevalence of type 2 DM parallels the increase in obesity[27] (hence the term “diabesity” by Ziv and Shafrir[28]) and reduced activity levels as countries become more industrialized, and with the aging of the population.

In 2010, the prevalence of diabetes ranged from 11.6 to 30.9%. 80% of the people with diabetes live in low and middle income countries. The ten countries with the highest prevalence in 2010 are Naurua, United Arab Emirates, Saudi Arabia, Mauritius, Bahrain, Reunion, Kuwait, Oman, Tona, Malaysia - in descending prevalence. There is variability in the prevalence between countries and between ethnic groups within a country owing to genetic, behavioural and environmental factors.

In the United States, diabetes was listed as the seventh leading cause of death in 2007; a recent estimate suggested that diabetes was responsible for almost four million deaths in 2010 (6.8% of deaths) and is the fifth leading cause of mortality worldwide[3], with cardiovascular complications of diabetes being the major cause of mortality.

INDIAN SCENARIO

India had 41 million diabetics in 2006, according to the International Diabetic Federation (IDF) and it is expected to increase to 70 million by the year 2025[2]. According to the World Health Organization (WHO) estimates, India had 32 million diabetic subjects in 2000, which is projected to increase to 80 million in 2030[29]. The 'Asian Indian phenotype' with greater degree of central obesity[30] as evidenced by greater waist circumference and waist hip ratios, greater visceral adiposity[31] ('thin fat

Indian babies')[32] and insulin resistance[33] contributes to increasing prevalence of type 2 diabetes in Asian Indians. Onset at a younger age is also noted in this group. The disease has also encroached upon the middle income and poorer sections of the Indian society and is no longer a disease of the affluent alone[34].The prevalence of coronary artery disease as a complication of diabetes was 21.4% as compared to 9.1% in subjects with normal glucose tolerance[35]. Also atherosclerosis, as assessed by carotid intimal thickness was higher in type 2 diabetics[36].The overall mortality rate was three fold higher(18.9 vs 5.3 per 1000 person-years) among diabetics as evidenced by Chennai Urban Population Study (CUPS) by Mohan V et al. Coronary artery disease appears to be the leading cause of death in majority of the studies[37-39].

CLASSIFICATION OF DIABETES

Diabetes mellitus is classified into type 1 and type 2 based on the pathogenic process leading to hyperglycemia. Complete or near-total insulin deficiency due to destruction of pancreatic beta cells results in type 1 DM. The much more prevalent type 2 DM is characterized by variable degrees of insulin resistance, insulin secretion and increased glucose production. Both are preceded by a phase of abnormal glucose homeostasis[5].

Etiologic classification of diabetes mellitus[5]

I. Type 1 diabetes (β-cell destruction, usually leading to absolute insulin deficiency)

A. Immune mediated

B. Idiopathic

II. Type 2 diabetes (may range from predominantly insulin resistance with relative insulin deficiency to a predominantly insulin secretory defect with insulin resistance)

III. Other specific types

A. Genetic defects of beta cell function characterized by mutations in Hepatocyte nuclear transcription factor (HNF)4α(MODY1); Glucokinase (MODY2); HNF- 1α(MODY3); Insulin promoter factor-1 (IPF-1;MODY4); HNF-1β (MODY5); Neuro D1(MODY6);Mitochondrial DNA; Subunits of ATP-sensitive potassium channel; Proinsulin or insulin

B. Genetic defects in insulin action

Type A insulin resistance; Leprechaunism; Rabson-Mendenhall syndrome; Lipodystrophy syndromes

C. Diseases of the exocrine pancreas- pancreatitis, pancreatectomy, neoplasia, cysic fibrosis, hemochromatosis, fibrocalculous pancreatopathy, mutations in carboxylester lipase

D. Endocrinopathies – acromegaly, Cushing's syndrome, pheochromocytoma, glucagonoma, hyperthyroidism, somatostatinoma, aldosteronoma

E. Drug-or chemical-induced – glucocorticoids, vacor (a rodenticide), pentamidine, nicotinic acid, diazoxide, β-adrenergic agonists, thiazides, hydantoins, asparaginase, α-interferon, protease inhibitors, antipsychotics(atypical and others), epinephrine

F. Infections – congenital rubella, cytomegalovirus, coxsackievirus

G. Uncommon forms of immune- mediated diabetes- 'stiff-person' syndrome, anti-insulin receptor antibodies

H. Other genetic syndromes sometimes associated with diabetes – Wolfram's syndrome, Down's syndrome, Klinefelter's syndrome, Turner's syndrome, Friedreich's ataxia, Huntington's chorea, Lawrence-moon-Biedl syndrome, myotonic dystrophy, porphyria, Prader-Willi syndrome

IV. Gestational diabetes mellitus (GDM)

DIAGNOSIS OF DIABETES MELLITUS

The three categories of glucose intolerance include normal glucose homeostasis, diabetes mellitus, and impaired glucose homeostasis.

1. Normal glucose tolerance – a fasting plasma glucose (FPG) < 100mg/dL (5.6mmol/L), a plasma glucose < 140 mg/dL (11.1 mmol/L) following an oral glucose challenge and an A1C <5.6%.

2. Diabetes mellitus

Criteria for the diagnosis of diabetes mellitus – American Diabetes Association (ADA) 2011[40]

A1C ≥ 6.5%. The test should be performed in a laboratory using a method that is NSPG certified and standardized to the DCCT assay.*

Or

FPG ≥ 126mg/dL (7.0mmol/L). Fasting is defined as no caloric intake for atleast 8 h.*

Or

2-h plasma glucose ≥200mg/dL (11.1mmol/L) during an OGTT. The test should be performed as described by the World Health Organization, using a glucose load containing the equivalent of 75g anhydrous glucose dissolved in water*

Or

In a patient with classic symptoms of hyperglycemia or hyperglycemic crisis, a random plasma glucose ≥ 200mg/dL (11.1mmol/L). Random is defined as without regard to time since the last meal.

*In the absence of unequivocal hyperglycemia and acute metabolic decompensation, result should be confirmed by repeat testing on a different day.

3. Abnormal glucose homeostasis

Categories of increased risk for diabetes (prediabetes)*[40]

FPG 100-125mg/dL (5.6-6.9mmol/L) - Impaired Fasting Glucose (IFG)

Or

2-h plasma glucose in the 75-g OGTT 140 - 199 mg/dL (7.8 - 11.0 mmol/L)-Impaired Glucose Tolerance (IGT)

Or

A1C 5.7-6.4%

*For all three tests, risk is continuous, extending below the lower limit of the range and becoming disproportionately greater at the higher ends of the range.

COMPLICATIONS OF DIABETES MELLITUS[5]

I. ACUTE

A. Diabetic ketoacidosis (DKA)

B. Hyperglycemic hyperosmolar state (HHS)

II. CHRONIC

A. VASCULAR

1. MICROVASCULAR

(A) Eye disease – Retinopathy (nonproliferative/proliferative)

- Macular edema

(B) Neuropathy – Sensory and motor (mono- and polyneuropathy)

- Autonomic

(C) Nephropathy

2. MACROVASCULAR

(A) Coronary heart disease

(B) Peripheral arterial disease

(C) Cerebrovascular disease

B. Other

1. Gastrointestinal (gastroparesis, diarrhoea)

2. Genitourinary (sexual dysfunction/uropathy)

3. Infectious

4. Hearing loss

5. Cataracts

6. Glaucoma

7. Dermatologic

8. Peridontal disease

MECHANISMS OF CHRONIC COMPLICATIONS OF DM

The majority of morbidity and mortality associated with diabetes are due to its chronic complications in multiple organ systems as mentioned above. The duration and degree of hyperglycemia determines the risk of chronic complications. They usually appear in the second decade of hyperglycemia. But owing to the long asymptomatic period of hyperglycemia in many individuals, they may present with complications at the time of diagnosis. Genetic susceptibility also plays a role[5].

The Diabetes Control and Complications Trial (DCCT)[41] and the United Kingdom Prospective Diabetes Study (UKPDS)[42] have established the causative role for chronic hyperglycemia in the pathogenesis of microvascular complications in both type1 and type2 diabetes mellitus, whereas for macro- vascular complications it is less conclusive. They also established that amelioration of hyperglycemia can modify this risk. In

individuals with type 2 DM, two to four times higher coronary heart disease events and mortality are noted, which correlates well with fasting and postprandial plasma glucose levels and A1C. Dyslipidemia and hypertension also contributes to the development of macrovascular disease in diabetics.

The vascular endothelial cells are unable to downregulate glucose uptake under hyperglycemic conditions, due to free passage of glucose through the endothelial cell membrane via the insulin-dependent glucose transporter GLUT-1 and hence they are more prone for developing intracellular hyperglycemia[43]. Four major biochemical pathways have been proposed to explain the association between chronic hyperglycemia and vascular damage.

A unifying hypothesis has been formulated whereby these four pathways can be linked by a single hyperglycemia - driven process: the mitochondrial overproduction of reactive oxygen species[44].

1. Formation of advanced glycosylation end products (AGEs)

The process of formation of AGEs by irreversible nonenzymatic glycosylation of intra- and extracellular proteins is markedly accelerated by chronic hyperglycemia[45]. AGEs crosslink proteins (collagen, extracellular matrix proteins), alter extracellular matrix structure and

composition, promote glomerular dysfunction, reduce nitric oxide synthesis, induce endothelial dysfunction and accelerate atherosclerosis.

2. Increased flux through the polyol pathway

In an individual with chronic hyperglycemia, an increase in glucose metabolism (upto 30% of glucose) via the sorbitol pathway occurs[46].The consumption of NADPH in the reduction of glucose to sorbitol by aldose reductase and subsequent oxidative stress is the most likely mechanism of damage[47,48]. Increased sorbitol concentration alters redox potential, increases cellular osmolality, generates reactive oxygen species and lead to cellular dysfunction.

3. Activation of protein kinase C(PKC)

Intracellular hyperglycemia has been shown to stimulate de novo synthesis of diacyl glycerol(DAG) .This increase in the DAG content in turn activates protein kinase C, principally β and δ isoforms[49]. Activated PKC leads to endothelial dysfunction (suppression of nitric oxide production and stimulation of endothelin-1 vasoconstrictor activity)[50], increased vascular permeability (via induction of vascular endothelial growth factor, VEGF)[51], accumulation of matrix proteins and mesangial expansion through induction of transforming growth factor β(TGF-β), type IV collagen, and fibronectin[52,53],and decreased fibrinolysis (via increased

plasminogen activator inhibitor-1, PAI-1)[54]. Finally, it also stimulate NADPH oxidases, generating reactive oxygen species (ROS), leading to increased oxidative stress.

4. Increased flux through hexosamine pathway

The hexosamine pathway may alter cellular function by O-linked glycosylation of proteins such as endothelial nitric oxide via UDP-N-acetyl glucosamine or by changes in gene expression of transforming growth factor β (TGF-β) or plasminogen activator inhibitor-1(PAI-1)[55-57].

CARDIOVASCULAR COMPLICATIONS IN DIABETES MELLITUS

There is an increased risk of cardiovascular disease in both types of diabetes mellitus. These account for the major mortality and morbidity in type 2 diabetics[58]. It is shown that the relative risk of cardiovascular disease is two to four fold higher in people with diabetes than in those without diabetes[59]. A marked increase in PAD, CHF, CAD, MI and sudden death (one- to five fold increase in risk) in DM was demonstrated in The Framingham Heart Study[5]. Uusitupa et al. demonstrated significantly higher age standardized prevalence of acute MI among newly diagnosed type 2 DM men (17%) and women (18%) as compared to non diabetics (10% and 4%respectively). Also, during the ten year follow up, the incidence of first MI was significantly higher among diabetic compared

with nondiabetic persons (7% vs.19% for men; 12% vs.2% for women) and also cardiovascular mortality[60]. The United Kingdom Prospective Diabetes Study (UKPDS), followed up patients with newly diagnosed type 2 diabetes over a 10-year period, and showed that 8.4% of type 2 diabetic patients died of either fatal MI or sudden death[42]. Excess mortality, CVD mortality in particular, extends beyond the limits of diabetes to impaired glucose tolerance (IGT) as evidenced by studies in the United Kingdom, in Whitehall[61], London and in Bedford[62]. Uusitupa et al. also showed that a considerable number of type2 DM patients had already suffered an MI before the time of diagnosis of their diabetes[63].

Diabetes confers an independent increased risk for cardiovascular complications. This is more so in the women ,in whom the diabetic status confers an increased risk ,equal to that of risk that is attributed to the overall male population [64,65]. The American Heart Association has designated DM as a "CHD risk equivalent"[5]. In a population based study conducted by Haffner SM et al, the 7-year incidence of first MI or death for patients with diabetes was 20% vs.3.5% for non diabetic patients. History of MI increased the rate of recurrent MI or cardiovascular death for both groups (18.8% in non diabetics and 45% in those with diabetes). Thus, the chance for a diabetic to get a first myocardial infarction is as high as that of a second infarction in a non diabetic person[66].

Diabetics have greater acute as well as subsequent mortality following an MI than nondiabetics[67] and have increased risk of reinfarction, congestive heart failure and death[68]. According to the FINMONICA study[69], the largest population based study of the impact of diabetes on mortality after the first MI, 1-year case-fatality rate for first MI (including prehospitalization mortality) was 45% in diabetic men and 39% in diabetic women as compared to 38% and 25% in non diabetics respectively. In short, in diabetics, CHD appears earlier in life, affects women almost as often as men, involve multiple vessels, and is more often fatal[70].

<u>In short cardiovascular complications of diabetes include</u>

I. Atherosclerosis

Potential atherogenic mechanisms include

(a) Lipid and lipoprotein aberrations

(b) Procoagulant state

(c) Hyperinsulinemia and insulin-resistance syndrome

(d) Glycation of proteins (AGEs)

(e) Inflammation

(f) Oxidative stress

(g) Microalbuminuria

II. Syndrome X

III. Congestive heart failure

IV. Autonomic neuropathy

V. Preoperative evaluation and post operative risks

Cardiovascular Risk factors associated with diabetes and insulin resistance[71]

A. Metabolic factors

1. Hyperglycemia
2. Insulin resistance
3. Hyperinsulinemia
4. Hypertriglyceridemia
5. Reduced HDL cholesterol
6. Small dense LDL
7. Hyperhomocysteinemia

B. Coagulation and inflammatory factors

1. Increased plasminogen activator inhibitor-1(PAI-1)

2. Increased platelet activation

3. Increased fibrinogen

4. Increased P-selectin, VCAM-1(vascular cell adhesion molecule-1), and ICAM-1(intracellular adhesion molecule-1)

5. Increased tissue factor and factor VII

6. Decreased nitric oxide bioavailabilty

7. Increased C-reactive protein

C. Vascular related factors

1. Hypertension

2. Impaired endothelium-dependent vasorelaxation

3. Increased arterial calcification

4. Decreased arterial compliance

The increase in cardiovascular mortality and morbidity rates in diabetes is due to the synergism of hyperglycemia with other cardiovascular risk factors (dyslipidemia, hypertension, obesity, reduced physical activity, cigarette smoking, microalbuminuria, macroalbuminuria, elevated serum creatinine, abnormal platelet function, insulin resistance and hyperinsulinemia, elevated levels of plasminogen activator inhibitor (PAI-1) and fibrinogen, endothelial and vascular smooth muscle dysfunction). Large benefits are seen when multiple risk factors are addressed globally[72,73].

TRADITIONAL CARDIOVASCULAR RISK FACTORS IN DIABETES

The traditional risk factors such as hypertension, dyslipidemia, obesity, reduced physical activity and cigarette smoking play an important role in the development of coronary heart disease in diabetic patients[65].

Hypertension:

Hypertension is a major CVD risk factor that often coexists with diabetes and insulin resistance[74]. The prevalence of hypertension in diabetic appears to be approximately twice as high as in non diabetics in the same population[75]. According to the joint data from Finnish and Dutch cohorts of the Seven Countries Study, glucose intolerance was proposed to be a stronger correlate of hypertension than is hyperinsulinemia[76]. Autooxidation of glucose[77], leading to the generation of oxygen-derived free radicals which in turn inactivate endothelium-derived releasing factors[78] and selectively attenuate endothelium dependent relaxation[79] contributes to elevated blood pressure in diabetics. The postprandial hypertriglyceridemia also have independent and cumulative deleterious effect on endothelial function through oxidative stress[80].

Epidemiologic analyses have revealed that in individuals with diabetes, blood pressure values >115/75 mmHg are associated with increased cardiovascular event rates and mortality[65,81,82]. A doubling in the

risk of CVD was shown for each incremental increase in BP of 20/10mmHg above 115/75 mmHg and for every 10mmHg reduction in systolic BP, and the risk of any diabetes-related complication decreased by 12%[83].The benefit of intensive blood pressure control in diabetics with regard to cardiovascular disease, stroke, retinopathy and nephropathy is also shown by various trials like The Appropriate Blood Pressure Control in Diabetes and the Hypertension Optimal Treatment (HOT)[84] .

According to these results, the American Diabetes Association along with AHA and ACC have set 130/80mmHg or lower as target BP in patients with diabetes[40,86]. The treatment of hypertension consists of lifestyle therapy and pharmacologic therapy[40]. Life style therapy includes weight loss in overweight individuals, Dietary Approaches to Stop Hypertension (DASH)-style dietary pattern including reducing sodium and increasing potassium intake; moderation of alcohol intake; and increased physical activity. Drug therapy is initiated with either ACE inhibitor or an ARB (shown to reduce the incidence of various cardiovascular end points in multiple studies)[87-91], with diuretics as the next add on therapy, usually requiring multiple drug therapy.

Dyslipidemia

The most common pattern of dyslipidemia in type 2 diabetes is decrease in HDL cholesterol level and an increase in the triglyceride. LDL cholesterol levels are usually normal . The importance is that the LDL particles in diabetics are more atherogenic[93] . This was mentioned as diabetic dyslipidema by Erkelens.[92]As part of comprehensive diabetes care, lipid abnormalities should be assessed and treated aggressively due to the additive cardiovascular risk of hyperglycemia and hypertriglyceridemia[5].

Analyzing subgroup of individuals with diabetes in various trials of primary prevention, statistically significant benefit with regard to CVD was found in the Heart Protection Study (HPS)[94,95] and Anglo-Scandinavian Cardiac Outcomes Trial-Lipid-Lowering Arm (ASCOT-LLA)[96] using statins. The Collaborative Atorvastatin Diabetes Study (CARDS), conducted with 10 mg atorvastatin in individuals with type 2 diabetes, showed a decrease in risk by 37% for cardiovascular events, and by 48% for stroke and 27% in total mortality[97].

Among the secondary prevention trials with diabetes subgroup analyses, statistically significant benefit with regard to cardiovascular event was noted in the Heart Protection Study(HPS)[94,95], the Scandinavian

Simvastatin Survival study(4S)[98], The Lescol Intervention Prevention Study(LIPS)[99], the Cholesterol and Recurrent Events (CARE)study[100], all using statins and with gembrozil in Veterans Administration High-Density Lipoprotein Cholesterol Intervention Trial(VA-HIT)[101] .

The current guidelines in the treatment of dyslipidemia in diabetics are provided by the ADA[40] and the AHA. The first priority is to lower the LDL cholesterol followed by raising the HDL cholesterol and decreasing the triglycerides. Initial therapy includes lifestyle modification (smoking cessation, increased physical activity, weight loss, blood pressure control) and dietary changes as advocated by the National Cholesterol Education Program to increase monounsaturated fats, carbohydrates, omega-3 fatty acids and viscous fibre and to reduce saturated fats and cholesterol. Improvement in glycemic control will lower triglycerides and raise HDL.

The target lipid values in diabetic individuals (>40years) without cardiovascular disease are LDL<100mg/dL (2.6mmol/L); HDL>40mg/dL (1mmol/L) in men and >50mg/dL (1.3mmol/L) in women; and triglycerides <150mg/dL (1.7mmol/L). The agents of choice for lowering the LDL level are HMG-CoA reductase inhibitors. In patients >40 years , in those with CHD and in those without CHD, but who have CHD risk factors, addition of a statin regardless of the LDL level is recommended. Reducing LDL cholesterol levels to 70 mg/dL was associated with a

reduction in cardiovascular events in studies like HPS[94,95], CARDS[97] and Incremental Decrease in End Points through Aggressive Lipid Lowering (IDEAL)[102]. In keeping with this, and the evidence in nondiabetic individuals with CHD, the ADA recommends an LDL goal of < 70mg/dL (1.8mmol/L) as an 'option' in patients with known CHD[5].

Smoking

Smokers have an increased rate of CVD, premature death, and increased rate of microvascular complications of diabetes and also may have a role in the development of type 2 diabetes. According to the ADA recommendations, all diabetic patients are advised not to smoke, and smoking cessation counselling, assessment of level of nicotine dependence and addition of pharmacological therapy in those motivated to quit should become a part of routine diabetes care[40].

Obesity

As many as 80% of individuals with type 2 diabetes are obese. Obesity is associated with other cardiovascular risk factors such as poorer glycemic control, higher blood pressures and atherogenic lipid profiles[106,107] (especially with abdominal obesity) and decreased levels of vascular protective adipokine-adiponectin; hence the need for active interventions to control weight gain. According to the Framingham study,

obesity (waist-to-hip ratio being the best predictor) was an independent risk factor for 26 year incidence of cardiovascular disease in men and women (including coronary disease, stroke and congestive heart failure). Weight loss is recommended for all overweight or obese individuals with type 2 diabetes as it is known to reduce insulin resistance. A low-carbohydrate, low-fat, calorie-restricted diet or Mediterranean diets may be effective in the short term (upto 2 years). Medical Nutrition Therapy (MNT) with a registered dietician is recommended.

Regular exercise also plays an important role in weight loss programs and are most helpful in maintenance of weight loss. It also improves blood glucose control and insulin sensitivity, reduce cardiovascular risk factors and improve well-being[103]. ADA recommends atleast 150min/week of moderate intensity aerobic physical activity (50-70% of maximum heart rate) and resistance training three times per week in the absence of contraindications (cardiovascular status should be taken into consideration)[40]. A reduction in the A1C level by an average of 0.66% was noted in type 2 diabetics with structured exercise interventions of atleast 8 weeks duration, even with no significant change in BMI[104,105].

Role of glycemic control

The Norfolk Cohort of the European Prospective Investigation of Cancer and Nutrition(EPIC-Norfolk) demonstrated that HbA1C was independently and continuously related to all-cause, cardiovascular and ischemic heart disease mortality throughout the whole population distribution. A 28% increase in the risk of death in men ($p<0.002$) independent of age, blood pressure, serum cholesterol, body mass index and cigarette smoking was noted with each 1% increase in HbA1C. An association between glucose and cardiovascular disease also existed at levels below those used to define diabetes[108]. ADA recommends an HbA1C <7% as the target for adequate glycemic control[40].

NOVEL CARDIOVASCULAR RISK FACTORS

The novel risk factors, shown to predict increased cardiovascular risk includes hsCRP and other markers of inflammation, lipoprotein(a), homocysteine, and markers of fibrinolytic and hemostatic function, such as fibrinogen, D-dimer, tissue plasminogen activator (t-PA), and plasminogen activator inhibitor 1 (PAI-1) antigens.

ATHEROSCLEROSIS AND DIABETES

Diabetes is associated with accelerated atherosclerosis. It has been established that hyperglycemia, oxidative stress, and inflammation play a

key role in atherogenesis as evidenced in diabetic vasculopathies. Libby and Ross have given evidence that all phases of atherosclerosis from the initiation of the fatty streak to the culmination in acute coronary syndromes (plaque rupture) involves inflammation[109,110] ('microinflammaion'). Diabetes is a pro-inflammatory state as evidenced by increased high-sensitivity C-reactive protein, fibrinogen, plasminogen activator inhibitor 1, pro-inflammatory cytokines, soluble cell adhesion molecules and nuclear factor κβ activity.

C-REACTIVE PROTEIN

CRP, an acute-phase reactant, a simple downstream marker of inflammation, has now emerged as a major cardiovascular risk factor. It is a member of the pentraxin family and has a cyclic pentameric structure[111]. It plays a major role in the human innate immune response. Its synthesis in the liver is triggered by various proinflammatory cytokines derived from various sources including monocytes, macrophages and adipose tissue. Studies have shown that cells in the human coronary artery particularly atherosclerotic intima can also produce CRP.

The oxidative stress and infectious agents such as Cytomegalovirus trigger a proinflammatory response in the vessel wall leading to increased secretion of interleukin-1β(IL-1β) and the proinflammatory cytokine

tumour necrosis factor α(TNF-α) which inturn releases cytokine IL-6, especially from the macrophages. IL-6, on binding to receptors in the liver causes secretion of CRP and Serum Amyloid A protein (SAA). CRP decreases endothelial nitric oxide production, increases release of adhesion molecules and PAI-1, promotes increased oxidized LDL uptake, increases release of ROS and proinflammatory cytokines[112].

The Pickup's group demonstrated that both IL-6 and high sensitivity-CRP are elevated in diabetic patients[113]. The Tan's group also demonstrated higher CRP levels in type 2 diabetic patients than matched non-diabetic controls[114]. Ford and co-workers in the NHANES III (National Health and Nutrition Examination Survey) noted that persons with diabetes or with impaired fasting glucose have increased levels of CRP compared with those who have normal fasting glucose[115]. High dietary glycemic load can also raise plasma concentrations of CRP, accounting for the increased level of CRP in type 2 DM[116]. The high CRP concentrations also appear to predict type 2 DM as evidenced by the Cardiovascular Health Study[117], the Women's Health Study[118], the MONICA Augsburg Cohort Study[119] and IRAS[120], supporting a role for chronic inflammation in the pathogenesis of diabetes.

Inflammation is also associated with many metabolic abnormalities associated with diabetes, especially with insulin resistance. The individuals

with type 2 diabetes with more than two features of the metabolic syndrome had more inflammation as evidenced by increased serum CRP and IL-6 levels[113]. In the Insulin Resistance and Atherosclerosis Study (IRAS), Festa and co-workers showed that hsCRP positively correlated with BMI, waist circumference, total cholesterol, triglycerides, LDL cholesterol, plasma glucose and fasting insulin. hsCRP was inversely correlated with HDL cholesterol and insulin sensitivity index. The strongest correlation of CRP was reported with BMI and insulin sensitivity index and a linear increase in CRP levels was noted with increase in the number of metabolic disorders[120]. Thus, hsCRP assessment also adds prognostic information at all levels of the metabolic syndrome [121,122]. So we can conclude that even for individuals with the Adult Treatment Panel (ATP-III) definition of metabolic syndrome, knowledge of hsCRP levels can predict risk groups for future vascular events.

In various studies involving primary prevention it is demonstrated that CRP, when measured with high sensitive assays (hsCRP) strongly and independently predicts risk of coronary vascular disease (CVD) including coronary artery disease, stroke, peripheral arterial disease and sudden death across all age levels, in both sexes and in diverse populations. Various studies have found the risk of hsCRP to be independent of and additive to traditional risk factors[123-126]. The magnitude of effect of hsCRP

is at least as large as that of hypertension and smoking, this underscores the importance of inflammation in atherogenesis[127]. hsCRP indicates an increased propensity for plaque disruption and/or thrombosis more than the presence of subclinical disease. Studies have shown that hsCRP levels correlate modestly with underlying atherosclerotic disease as measured by carotid intimal medial thickness[129] or by coronary calcification. hsCRP levels also predict incident hypertension and add prognostic information on vascular risk at all levels of blood pressure[129,130]. hsCRP levels have been proved to have strong predictive value for poor short- and long-term cardiovascular outcomes in allograft atherosclerosis[132] and chronic renal failure and dialysis[131].

Most importantly, hsCRP adds prognostic information at all levels of LDL cholesterol and at all levels of risk, as determined by the Framingham Risk Score[133]. Some others have shown that hs-CRP is a better predictor of subsequent risk than LDL cholesterol level itself [134,135]. Since hsCRP and cholesterol reflect different components of vascular risk, the addition of hsCRP to lipid evaluation provides a greater opportunity to improve global risk prediction. The absolute vascular risk is higher in individuals with elevated hsCRP levels and low levels of LDL cholesterol than in those with elevated levels of LDL cholesterol but low levels of

hsCRP, but current guidelines consider only the latter group to be at high risk.

The guidelines in 2003 issued by the American Heart Association and the Centres for Disease Control and Prevention regarding the use of hsCRP in clinical practice[136] interprets hsCRP levels less than 1, 1 to 3, and higher than 3 mg/L as lower, moderate, and higher relative vascular risk, respectively, when considered along with traditional markers of risk. Its greatest usefulness is likely to be for those at intermediate risk—that is, individuals with anticipated 10-year event rates between 5 and 20 percent, although hsCRP predicts risk across the entire population spectrum. The global risk prediction models that included hsCRP reclassified approximately 20 percent of those individuals otherwise considered to be at intermediate risk. In recent studies, it is shown that the impact of hsCRP on risk prediction was at least as large as that of lipid screening [137]. Recent studies have demonstrated that the Reynolds Risk Score combining hsCRP and information on family history of coronary heart disease (history of MI in a parent before 60 years) with the Framingham risk factors more accurately estimate cardiovascular risk in both men and women. Newer guidelines by the Canadian Cardiovascular Society in 2009, after taking into consideration the results of Justification for the Use of Statins in

Prevention And Intervention Trial Evaluating Rosuvastatin (JUPITER)[138] call for measurement of hsCRP in all patients with intermediate risk.

Screening for hsCRP is not to be considered as a replacement for LDL and HDL testing. Rather it should be done at the discretion of the physician as part of global risk evaluation on an outpatient basis at the time of cholesterol evaluation. hsCRP levels are stable over long periods, have no circadian variation, and do not depend on prandial state. The cardiovascular risk appears to be linear across the full range of hsCRP levels[126]. hsCRP values more than 8 mg/L may represent an acute-phase response caused by an underlying inflammatory disease or intercurrent infection; but consistently high values, as evidenced by repeat testing approximately two to three weeks later, however, represent very high risk of future cardiovascular disease .

Levels of hsCRP greater than 3 mg/L also has prognostic role in acute ischemia even without troponin level elevation[139], and predicts poor outcome in the setting of unstable angina, recurrent coronary events, thrombotic complications after angioplasty, and vascular complications after bypass surgery in those with established coronary heart disease. Hence individuals with elevated hsCRP levels are also more likely to benefit from aggressive interventions compared with those with low hsCRP levels.

Statins lower hsCRP levels in a manner largely unrelated to the magnitude of LDL cholesterol reduction[141]. Statin therapy prevents first vascular events, not only in patients with elevated LDL cholesterol levels, but also in those with elevated levels of hsCRP as demonstrated in AFCAPS/TexCAPS trial [140]. The concept of dual goals for statin therapy which includes both CRP and LDL reduction has emerged with the results of the PROVE IT-TIMI 22 clinical trial conducted in patients with acute coronary syndromes treated with statin therapy; the best long-term outcomes were found in those who achieved reduction of hsCRP less than 2mg/L and LDL cholesterol less than 70 mg/dL[142].

The recent JUPITER trial showed that statin use in healthy men aged > 50 years and in healthy women aged > 60 years with an LDL of < 130 mg/dL and an hsCRP level of > 2 mg/L decreased the incidence of a first major cardiovascular event (eg, MI, stroke, arterial revascularization procedure, hospitalization with unstable angina, or death from cardiovascular causes) by 44% ($P < 0.001$). A 55% reduction was observed for the end point of MI, whereas for stroke, a 48% reduction was observed. For the prevention of one event, the estimated number needed to treat for 2 and 4 year was 95 and 31, respectively[143], which makes this approach more efficient than the treatment of either hyperlipidemia or hypertension in similar primary prevention patients. In a recently published subanalysis of

JUPITER, Ridker et al showed that the best cardiovascular outcomes occurred in patients who attained an LDL-C of < 70 mg/dL and an hsCRP level of < 1 mg/L with statins[138]. Treatment with fibrates, niacin, thiazolodinediones may also lower hsCRP levels. The usefulness of aspirin in preventing first vascular events appears to be highest for patients with elevated hsCRP levels, even though aspirin has not shown to reduce hsCRP levels. This suggests individuals with high hsCRP levels may benefit from the use of aspirin for primary prevention.

Current strategies recommend aggressive primary prevention in those individuals with LDL cholesterol >160 mg/dL and elevated hsCRP levels. This may also motivate some patients to comply with life style modifications and pharmacotherapy. In those with LDL cholesterol levels between 130 and 160mg/dl, the finding of an elevated hsCRP level indicates substantial risk and, again, should lead to better adherence to preventive efforts and perhaps to earlier use of pharmacological approaches to risk reduction. For individuals with LDL cholesterol levels below 130 mg/dl, an elevated hsCRP value also confers elevated risk. Such individuals should thus aggressively undergo lifestyle modification (physical activity, weight loss, and smoking cessation programs) with earlier pharmacotherapy. As noted earlier, in patients with acute coronary syndromes, reaching an hsCRP level less than 2 mg/L appears to be as

important for long-term outcomes as reaching an LDL cholesterol level less than 70 mg/dl. The newer guidelines, such as those issued in 2009 in Canada, now list an hs-CRP level of < 2 mg/L as a secondary target for statin therapy.[144]

DYSREGULATION OF HEMOSTASIS IN DIABETES

Both diabetes and insulin resistance are prothrombotic states. Disturbances of the hemostatic system favour the development of vascular damage and thrombosis at the site of an acutely ruptured atherosclerotic plaque. Changes in the hemostatic system seen in diabetes include hyperactivity of platelets, high levels of fibrinogen, which promotes platelet aggregation and induces rheological changes and the formation of a rich fibrin clot, activation of endothelial cells and leucocytes, hypercoagulabilty with increased formation of multipotent thrombin and decreased fibrinolytic activity due to an increased plasma level of Plasminogen activator inhibitor-1(PAI-1)

Hyperglycemia per se through the formation of AGEs release proinflammatory cytokines which increases cell surface adhesion molecules such as vWF, decrease production of nitric oxide, promotes oxidative stress, forms glycated fibrin and glycated platelet glycoproteins with altered function.

FIBRINOGEN

Fibrinogen, like CRP, is an acute-phase reactant and increases during inflammatory responses. Fibrinogen was among the first "novel" cardiovascular risk factors to be evaluated. Also known as clotting factor I, it is a soluble glycoprotein synthesized in the liver. The fibrinogen is an asymmetrical molecule, which is highly elongated having an axial ratio of 20:1, the asymmetry and large size of which contributes to the viscosity of blood[145].

Plasma fibrinogen influences platelet aggregation and blood viscosity, interacts with plasminogen binding and, in combination with thrombin, mediates the final step in clot formation and the response to vascular injury[146]. In addition, fibrinogen associates positively with age, obesity, smoking, diabetes and glycemic control, and LDL cholesterol level, and inversely with HDL cholesterol level, alcohol use, physical activity, and exercise level.

Several studies have shown that fibrinogen levels are increased in type 2 DM patients and they are closely related with the presence of vascular complications[147,148]. Kannel B et al, in his study showed that there was an increase in fibrinogen values throughout the range of blood sugar levels[149]. Graziella Brino et al studied the association of serum fibrinogen

level with glycemic control in 1525 patients and found that noninsulin dependent diabetic patients had a high prevalence of hyperfibrinogenemia and that fibrinogen level was independently associated with HbA1c value[150]. Hyperfibrinogenemia in diabetes has been reported to be caused by an increased synthesis of fibrinogen that is not compensated for by a proportional increase in clearance of fibrinogen. These abnormalities have been associated with insulin deficiency, suggesting that hyperfibrinogenemia is an expression of poor glycemic control.

In review of risk factors for coronary artery disease Jarrett RJ has mentioned that increased fibrinogen, lipoprotein abnormalities and increased platelet adhesiveness contribute to the same[151]. Graziella Bruno et al[150], Kannel W B et al[149], Lee AJ[152] et al have also found an association between increased plasma fibrinogen level and increased cardiovascular risk in diabetic patients. Also the Gothenburg, Northwick Park[153], and Framingham heart studies all found significant positive associations between fibrinogen levels and future risk of cardiovascular events and that there was an approximately linear logarithmic association between usual fibrinogen level and the risk of coronary heart disease and stroke, independent of hsCRP levels. In one analysis, the age- and gender-adjusted hazard ratio per 1-g/L increase in fibrinogen was 2.4 for coronary heart disease and 2.1 for stroke.[154]

In more recent studies, hsCRP and fibrinogen levels appeared to be additive in their ability to predict risk, although the absolute effect of hsCRP appeared to be larger. Also there are studies to suggest that the predictive usefulness of fibrinogen is highest in those with other concomitant elevations of lipoprotein(a) or homocysteine.

It has been observed that fibrates and niacin lower fibrinogen levels but statin therapy does not, an effect different than that observed for hsCRP. The potential benefits of fibrinogen reduction has to be studied further. The Bezafibrate Infarction Prevention Trial did not show significant reduction in event rates with active therapy despite a significant reduction in fibrinogen levels and despite evidence that within the study population, baseline fibrinogen levels predicted vascular risk.[155]

MATERIALS AND METHODS

SETTING

Department of Internal Medicine

Government Stanley Medical college and Hospital

Chennai – 600 001

INSTITUTIONAL ETHICS COMMITTEE APPROVAL

Obtained

STUDY DESIGN

To assess the level of hsCRP and plasma fibrinogen level as a predictor of cardiovascular risk in individuals with type 2 diabetes mellitus, and its relationship to glycemic status, an observational case-control study was chosen.

PERIOD OF STUDY

May 2011 to November 2011

SAMPLE SIZE

Cases : 50 ; Controls : 50

INCLUSION CRITERIA

Diagnosis of Diabetes Mellitus according to criteria put forward by American Diabetic Association (ADA) - Standards of Medical care in Diabetes 2011.

Diabetic patients were classified as type 2 using the following parameters

: age of onset – after 30 years.

: those who did not require insulin therapy initially.

EXCLUSION CRITERIA

1. Diabetic patients with clinical evidence of neuropathy, nephropathy, retinopathy
2. Diabetic ketoacidosis and nonketotic hyperglycemic coma
3. Coronary artery disease
4. Peripheral vascular disease
5. Cerebrovascular disease
6. Liver disease
7. Renal disease
8. Pregnant diabetics
9. Acute infections
10. Smokers and alcoholics
11. Patients above the age of 50 yrs (to exclude asymptomatic ischemic heart disease)

STUDY PROTOCOL

A total of 100 patients who attended our outpatient clinic at the Government Stanley Medical College and Hospital were enrolled into the study. Of these fifty patients who met the criteria were selected as cases and 50 patients who came for Master health checkup were selected as controls.

The patients with the following criteria (according to the ADA-Standards of Medical Care in Diabetes 2011) were defined as having diabetes mellitus:

A1C ≥ 6.5%. The test should be performed in a laboratory using a method that is NSPG certified and standardized to the DCCT assay.*

Or

FPG ≥ 126mg/dL(7.0mmol/L). Fasting is defined as no caloric intake for atleast 8 h.*

Or

2-h plasma glucose ≥200mg/dL(11.1mmol/L) during an OGTT. The test should be performed as described by the World Health Organization, using a glucose load containing the equivalent of 75g anhydrous glucose dissolved in water*

Or

In a patient with classic symptoms of hyperglycemia or hyperglycemic crisis, a random plasma glucose ≥ 200mg/dL(11.1mmol/L). Random is defined as without regard to time since the last meal.

*In the absence of unequivocal hyperglycemia and acute metabolic decompensation, result should be confirmed by repeat testing on a different day.

Diabetic patients were classified as type 2 using the following parameters:

1. Age of onset-after 30 years.

2. Those who did not require insulin therapy initially.

For each enrolled subject, an informed written consent was taken. A detailed history was taken from each patient including details regarding the diagnosis, duration of diabetes, type of treatment, followup and other comorbidities. The duration of diabetes was not given greater emphasis as asymptomatic hyperglycemia persists in many much before the detection of their diabetic status.A complete physical examination of each patient was done which included search for peripheral vascular disease, cerebrovascular disease, cardiovascular disease, neuropathy, retinopathy and nephropathy.

Blood pressure was measured in the right arm in the sitting position with the arm supported using a mercury sphygmomanometer to the nearest 2mmHg, and repeated after 5 minutes rest if the first reading was high. Height was measured to the nearest mm with a stadiometer. Weight was measured and Body mass index was calculated using the following formula –

$$BMI = \text{Weight in Kgs} / (\text{Height in Ms})^2$$

Basic blood investigations including hemoglobin, total and differential counts, platelet counts, blood urea, serum creatinine, and liver function tests were done. Urine albumin, sugar, microscopy and ketone body estimation were done. A standard 12-lead resting electrocardiography and X-ray screening of chest and echocardiography was done. A fasting lipid profile including serum total cholesterol, triglycerides, high density lipoprotein(HDL), low density lipoprotein(LDL) was obtained. Fasting and 2-hour postprandial blood samples were obtained. Glycosylated hemoglobin(HbA1C) was done by Biorad HPLC assay. Normal value was taken as 4-6%. Patients with HbA1C $\geq$ 8% were categorized as those having poor control.

Plasma fibrinogen was estimated by automated optical light scattering method. Normal range is 180-350mgs/dL. High sensitive C-

reactive protein (hsCRP) was measured by immunoturbidometry and values more than 3.0 mg/L accounted for high cardiac risk. Values more than 10mg/L is suggestive of other inflammatory diseases or infection. The study group was compared with an age and sex matched control group of non diabetics .The results were recorded in a standard proforma.

STATISTICAL ANALYSIS

Statistical analysis was done in all the 100 subjects (50 cases and 50 controls) after categorizing the variable. Age, body mass index(BMI), systolic blood pressure, diastolic blood pressure, serum total cholesterol, triglycerides, LDL cholesterol, HDL cholesterol, fasting blood sugar, 2-hour postprandial blood sugar, HbA1C , plasma fibrinogen, hsCRP of all subjects were the parameters analysed. Statistical analysis was carried out with SPSS software. Pearson correlation coefficient and students t test were done to find out correlations and significant differences between the two groups. Statistical significance was taken when the p value as <0.05.

OBSERVATIONS AND RESULTS

In this study there were 50 patients in the study group which included 27 males and 23 females. The control group also had 50 subjects, ie, 22 males and 28 females.

AGE

In the study group, age of the patients ranged from 32 to 49 years, with a mean and standard deviation of 41.10 and 4.00 respectively. The majority of the patients belonged to the age group 40-44 years (56%).

Among the controls, age ranged from 30 to 49 years. The mean and standard deviation were 40.04 and 4.28 respectively. Majority were in the age group 40-44 years (52%).

Table – 1 Distribution of cases and controls by age group

Age in Years	Study group (n = 50)		Control group (n =50)	
	Number	Percentage	Number	Percentage
30-34	3	6.00	4	8.00
35-39	14	28.00	16	32.00
40-44	28	56.00	26	52.00
45-49	5	10.00	3	6.00

BODY MASS INDEX

The BMI of the study group ranged from 21.51 to 30.82kg/m^2. The mean and standard deviation were 25.10 and 1.67 respectively.

Among the controls, BMI varied from 21.64 to 27.06 kg/m^2 with a mean of 24.18 and a standard deviation of 1.43.

Table 2- Distribution of subjects in study group according to Body Mass Index

BMI(kg/m^2)	Number (n = 50)	Percentage
<18.5	0	0
18.5 – 24.9	28	56
25 – 29.9	21	42
30 -34.9	1	2

Table 3- Distribution of subjects in control group according to Body Mass Index

BMI (kg/m^2)	Number (n=50)	Percentage
<18.5	0	0
18.5 – 24.9	37	74
25 – 29.9	13	26
30 – 34.9	0	0

HYPERTENSION

Among the 50 subjects in the study group, 32(64%) were found to be hypertensive and the remaining 18(36%) were non hypertensives. Of the 32 hypertensive, 15 were males and 17 were females.

Among the 50 subjects in the control group, 8(16%) were hypertensives and 42(84%) were non hypertensives. Of the 8 hypertensives, 4 were males and 4 were females.

Table 4 – Hypertension in the study group and control group

	Study group (n=50)				**Control group(n=50)**			
	Males	**Females**	**Total**	**%**	**Males**	**Females**	**Total**	**%**
Hypertensives	15	17	32	64	4	4	8	16
Non-hypertensives	12	6	18	36	18	24	42	84

In the study group, the systolic blood pressure ranged from 110 to 160 mmHg with a mean value of 135.04 mmHg and a standard deviation of 13.28 mmHg.

In the control group, the systolic blood pressure value varied from 70 to 100mmHg, with a mean value of 128.40 mmHg and a standard deviation of 10.91 mmHg.

In the study group, the diastolic blood pressure ranged from 110 to 158 mmHg, with a mean value of 82.16 mmHg and a standard deviation of 8.30 mmHg.

In the control group, the diastolic blood pressure ranged from 70 to 100 mmHg, with a mean value of 78.56 mmHg and a standard deviation of 8.26 mmHg.

LIPID PROFILE

SERUM TOTAL CHOLESTEROL

In the study group, the value of serum total cholesterol ranged between 155 to 293 mg/dL with a mean value of 215.70 mg/dL and a standard deviation of 31.00 mg/dL.

In the control group, the value ranged from 162 to 240 mg/dL with a mean value of 185.12 mg/dL and a standard deviation of 23.45mg/dL.

TRIGLYCERIDES

In the study group, the value of triglycerides ranged from 130 to 270 mg/dL with a mean value of 184.42 mg/dL and a standard deviation of 31.86 mg/dL.

In the control group, the value ranged from 108 to 200 mg/dL with a mean value of 148.00 mg/dL and a standard deviation of 21.42 mg/dL.

HIGH DENSITY LIPOPROTEIN (HDL)

In the study group, HDL value ranged from 28 to 59 mg/dL with a mean value of 38.54 mg/dL and a standard deviation of 9.02 mg/dL.

In the control group, HDL value ranged from 35 to 60mg/dL with a mean value of 49.60 mg/dL and a standard deviation of 8.65 mg/dL.

LOW DENSITY LIPOPROTEIN (LDL)

In the study group, LDL values ranged from 75 to 210 mg/dL with a mean value of 140.24 mg/dL and a standard deviation of 33.36 mg/dL.

In the control group, LDL values ranged from 73 to 168 mg/dL with a mean value of 104.62 mg/dL and a standard deviation of 27.11 mg/dL.

BLOOD GLUCOSE

The study group had fasting blood glucose of 138.06 ± 17.13 mg/dL and 2-hour postprandial blood glucose of 223.12 ± 30.63 mg/dL.

In the control group, the values were 85.34±6.75mg/dL and 112.64±26.76 mg/dL respectively.

GLYCEMIC CONTROL

Glycemic control was measured by using the level of HbA1C (glycosylated haemoglobin) in blood. In the study group, HbA1C varied

from 6.6 to 9.2 gm/dL with a mean and standard deviation of 7.97 and 0.85 gm/dL respectively.

In the control group, the value ranged from 4 to 5.5 gm/dl with a mean of 4.94 and a standard deviation of 0.39gm/dL. Patients in the study group were divided into two groups based on the level of HbA1C. 28 patients had HbA1c≥8 gm/dL, implying poor glycemic control.

Table 5- Distribution of cases according to glycemic control

HbA1C(gm/dL)	Control of blood glucose	Number	Percentage
<8	Good	22	44
≥8	Poor	28	56

Table 6- Clinical and Biochemical Characteristics Of Study Subjects

	STUDY GROUP (n=50)	CONTROL GROUP (n=50)	P value
Age	41.10 ± 4.00	40.04 ± 4.28	0.204
BMI	25.10 ± 1.67	24.18 ± 1.43	0.004
SBP	135.04 ± 13.28	128.40 ± 10.91	0.007
DBP	82.16 ± 8.30	78.56 ± 8.261	0.032
T CH	215.70 ± 31.00	185.12 ± 23.45	<0.001
TG	184.42 ± 31.86	148.00 ± 21.42	<0.001
HDL	38.54 ± 9.02	49.60 ± 8.65	<0.001
LDL	140.24 ± 33.36	104.62 ± 27.11	<0.001
FBS	138.06 ± 17.13	85.34 ± 6.75	<0.001
PPBS	223.12 ± 30.63	112.64 ± 26.76	<0.001
HbA1C	7.97 ± 0.85	4.94 ± 0.39	<0.001

Both the study and control groups were comparable with respect to age (p 0.204). The study group had higher body mass index(p 0.004), higher systolic blood pressure(p 0.007), higher diastolic blood pressure(p 0.032), higher serum total cholesterol ($p<0.001$), higher triglyceride levels($p<0.001$), higher LDL cholesterol($p<0.001$), lower HDL cholesterol ($p<0.001$), higher fasting blood glucose ($p<0.001$), higher 2 –hour postprandial blood glucose ($p<0.001$) and higher HbA1C levels ($p<0.001$).

hsCRP

The mean hsCRP level in type 2 DM subjects was 3.41 mg/L and standard deviation is 1.16 mg/L. In the control group, the value was 2.35mg/L and standard deviation is 0.65mg/L.

For 39 diabetic patients, hsCRP was > 3.0 mg/dL (high risk).

PLASMA FIBRINOGEN

In the study group, plasma fibrinogen ranged from 276 to 463 mg/dL, the mean and standard deviation being 387.84 mg/dL and 57.11mg/dL respectively.

In the control group, the fibrinogen level varied from 170 to 376 mg/dl with a mean and standard deviation of 300.06 mg/dL and 50.59 mg/dL respectively.

In 43 diabetic patients, the plasma fibrinogen level was above the upper limit of normal (>350 mg/dl).

Table-7 Comparative Analysis Of hsCRP Level In The Study And The Control Groups:

	N	Mean	SD	Std. error of mean	't' value
Study group	50	3.41	1.16	0.16	5.571
Control group	50	2.35	0.65	0.09	

The p value is < 0.001. There was a highly significant positive correlation between diabetic state and the hsCRP level.

Table-8 Comparative Analysis Of Plasma Fibrinogen Level In The Study And Control Groups

	N	Mean	SD	Std error of mean	't' value
Study group	50	387.84	57.11	8.08	8.135
Control group	50	300.06	50.59	7.16	

The p value is <0.001. There was a highly significant positive correlation between diabetic state and the plasma fibrinogen level.

Table-9 Correlation Analysis Between Age, Body Mass Index, Systolic Blood Pressure, Diastolic Blood Pressure And hsCRP In The Study Group

Variables	N	Pearson Correlation ('r')	P value
Age	50	0.187	0.194
BMI	50	0.577	<0.001
Systolic BP	50	0.573	<0.001
Diastolic BP	50	0.598	<0.001

In the study group, a statistically significant positive correlation (p value <0.001) was found between Body Mass Index, systolic blood pressure, diastolic blood pressure and hsCRP. There was no significant correlation between age of the subject and hsCRP level.

Table-10 Correlation Analysis Between Age, Body Mass Index, Systolic Blood Pressure, Diastolic Blood Pressure And Hscrp In The Control Group

Variables	N	Pearson Correlation ('r')	P value
Age	50	0.518	<0.001
BMI	50	0.653	<0.001
Systolic BP	50	0.587	<0.001
Diastolic BP	50	0.529	<0.001

In the control group, a statistically significant positive correlation (p value <0.001) was found between Body Mass Index, systolic blood pressure, diastolic blood pressure and hsCRP. There was also significant correlation between age of the subject and hsCRP level.

Table 11- CORRELATION ANALYSIS BETWEEN LIPID PROFILE AND hsCRP IN THE STUDY GROUP

Variables	N	Pearson correlation('r')	p value
Serum total cholesterol	50	0.671	<0.001
Triglyceride	50	0.631	<0.001
HDL	50	0.765	<0.001
LDL	50	0.708	<0.001

In the study group, a statistically significant positive correlation (p value <0.001) was observed between serum total cholesterol, triglyceride, HDL, LDL and hsCRP.

Table 12- Correlation Analysis Between Lipid Profile And hsCRP In the control group

Serum total cholesterol	50	0.777	<0.001
Triglyceride	50	0.764	<0.001
HDL	50	0.791	<0.001
LDL	50	0.748	<0.001

A statistically significant positive correlation (p value <0.001) was noted between serum total cholesterol, triglyceride, HDL, LDL and hsCRP in the control group.

Table-13 Correlation Analysis Between Fasting Blood Glucose, 2-Hour Postprandial Blood Glucose, Hba1c And Hscrp Level In The Study Group

Variables	N	Pearson correlation(‘r’)	p value
Fasting blood glucose	50	0.853	<0.001
Postprandial blood glucose	50	0.807	<0.001
HbA1C	50	0.937	<0.001

In the study group, a statistically significant correlation with a p value <0.001 was noted between fasting blood glucose, 2-hour postprandial blood glucose and hsCRP level. Also a statistically significant positive correlation (p value<0.001) was found between Glycosylated hemoglobin (HbA1C) and hsCRP in the study group.

Table-14 Correlation Analysis Between Fasting Blood Glucose, 2-Hour Postprandial Blood Glucose, HbA1C and hsCRP Level In The Control Group

Variables	N	Pearson correlation(‘r’)	p value
Fasting blood glucose	50	0.223	0.119
Postprandial blood glucose	50	0.003	0.981
HbA1C	50	0.073	0.612

There was no statistically significant correlation between fasting blood glucose, 2-hour postprandial blood glucose and hsCRP in the control group. And there was no statistically significant correlation between HbA1C and hsCRP in the control group.

Table-15 Correlation Analysis Between Age, Body Mass Index, Systolic Blood Pressure, Diastolic Blood Pressure And Plasma Fibrinogen In The Study Group

Variables	N	Pearson Correlation (‘r’)	P value
Age	50	0.215	0.134
BMI	50	0.553	<0.001
Systolic BP	50	0.568	<0.001
Diastolic BP	50	0.567	<0.001

In the study group, a statistically significant positive correlation with a p value of <0.001 was found between Body Mass Index, systolic blood pressure, diastolic blood pressure and plasma fibrinogen. There was no statistically significant correlation between age of the subject and plasma fibrinogen.

Table-16 Correlation Analysis Between Age, Body Mass Index, Systolic Blood Pressure, Diastolic Blood Pressure And Plasma Fibrinogen In The Control Group

Variables	N	Pearson Correlation ('r')	P value
Age	50	0.512	<0.001
BMI	50	0.583	<0.001
Systolic BP	50	0.523	<0.001
Diastolic BP	50	0.454	<0.001

In the control group, a statistically significant positive correlation was observed between Body Mass Index, systolic blood pressure, diastolic blood pressure, and plasma fibrinogen with p value of <0.001. Age also significantly correlated.

Table-17 Correlation Analysis Between Lipid Profile And Plasma Fibrinogen In The Study Group

Variables	N	Pearson correlation (‘r’)	p value
Serum total cholesterol	50	0.641	<0.001
Triglyceride	50	0.601	<0.001
HDL	50	0.705	<0.001
LDL	50	0.672	<0.001

In the study group, a statistically significant positive correlation with a p value of <0.001 was found between serum total cholesterol, triglycerides, HDL, LDL and plasma fibrinogen.

Table 18- Correlation Analysis Between Lipid Profile And Plasma Fibrinogen In The Control Group

Variable	N	Pearson correlation(‘r’)	p value
Serum total cholesterol	50	0.705	<0.001
Triglyceride	50	0.700	<0.001
HDL	50	0.699	<0.001
LDL	50	0.673	<0.001

In the control group, a statistically significant positive correlation was observed between serum total cholesterol, triglycerides, HDL, LDL and plasma fibrinogen with p value of<0.001.

Table-19 Correlation Analysis Between Fasting Blood Glucose, 2-Hour Postprandial Blood, Hba1c And Plasma Fibrinogen Level In The Study Group

Variables	N	Pearson correlation(‘r’)	p value
Fasting blood glucose	50	0.866	<0.001
Postprandial blood glucose	50	0.815	<0.001
HbA1C	50	0.973	<0.001

In the study group, a statistically significant positive correlation with a p value of <0.001 was found between fasting blood glucose, 2-hour postprandial blood glucose and plasma fibrinogen. In the study group, statistically significant positive correlation with a p value of <0.001 was observed between Glycosylated haemoglobin (HbA1C) and plasma fibrinogen.

Table-20 Correlation Analysis Between Fasting Blood Glucose, 2-Hour Postprandial Blood Glucose And Plasma Fibrinogen Level In The Control Group

Variable	N	Pearson correlation (‘r’)	p value
Fasting blood glucose	50	0.231	0.107
Postprandial blood glucose	50	0.015	0.919
HbA1C	50	0.150	0.300

There was no statistically significant correlation fasting blood glucose, 2-hour postprandial blood glucose and plasma fibrinogen in the control group. There was no statistically significant correlation between Glycosylated hemoglobin level and plasma fibrinogen level in the control group.

Table-21 Correlation Analysis Between Hscrp And Plasma Fibrinogen In The Study Group

Variable	N	Pearson correlation (‘r’)	p value
hsCRP	50	0.971	<0.001

In the study group, statistically significant positive correlation with a p value of <0.001 was found between hsCRP and plasma fibrinogen.

Table-22 Correlation Analysis Between Hscrp And Plasma Fibrinogen In The Control Group

Variable	N	Pearson correlation (‘r’)	p value
hs-CRP	50	0.981	<0.001

A statistically significant correlation (p value <0.001) was observed between hsCRP and plasma fibrinogen in the control group.

Table-23 Correlation Between Hscrp And Plasma Fibrinogen Level With Glycemic Control In Diabetics

Variable	HbA1C<8 (n= 72)	HbA1C≥8 (n= 28)	t value	P value
Fibrinogen (mean ± SD)	310.29±48.93	430.50± 24.80	12.370	<0.001
hs CRP (mean ± SD)	2.36±0.39	4.20±0.78	11.90	<0.001

In the study group plasma fibrinogen level was higher in patients with poor glycemic control (HbA1C ≥ 8 gm/L) (430.50 ± 24.80) than in those with good glycemic control (HbA1C < 8gm/L) (310.29 ± 48.93) with a significant p value <0.001.

In the study group hsCRP level was also higher in diabetics with poor glycemic control (HbA1C ≥ 8 gm/L) (94.20 ± 0.78) than in those with good glycemic control (HbA1C < 8gm/L) (2.36 ± 0.39). The p value was significant (<0.001).

Table-24 Clinical And Biochemical Characteristics In Normal And Abnormal Hs-Crp Levels

PARAMETERS	Normal hs-CRP (hs-CRP <3.0) (N = 11)	Abnormal hs-CRP (hs-CRP >3.0) (N = 39)	t	p value
Age(years)	40.82 ± 2.86	41.18 ± 4.30	-0.262	0.794
BMI(kg/m^2)	23.77 ± 1.58	25.47 ± 1.51	-3.272	0.002
Systolic BP(mmHg)	125.82 ± 12.02	137.64 ± 12.57	-2.781	0.008
Diastolic BP(mmHg)	76.18 ± 8.36	83.85 ± 7.56	-2.903	0.006
Total cholesterol (mg/dL)	186.45 ± 34.66	233.95 ± 24.61	-4.065	<0.001
Triglycerides(mg/dL)	153.55 ± 27.39	193.13 ± 27.53	-4.216	<0.001
HDL(mg/dL)	48.91 ± 9.80	35.62 ± 6.30	-5.428	<0.001
LDL(mg/dL)	107.09 ± 38.09	149.59 ± 25.40	-4.366	<0.001
Fasting blood glucose(mg/dL)	116.27 ± 12.38	144.21 ± 12.72	-6.468	<0.001
Postprandial blood glucose(mg/dL)	185.91 ± 22.88	233.62 ± 23.63	-5.953	<0.001
HbA1C	6.80 ± 0.25	8.29 ± 0.65	-7.353	<0.001

When the study subjects were characterised as high risk using hsCRP cut-off >3.0 mg/L, the subjects with abnormal hsCRP (3.0mg/L) had higher BMI (p 0.002), higher systolic blood pressure(p 0.008), higher diastolic blood pressure (p 0.006), higher serum total cholesterol (p<0.001), higher triglyceride levels (p<0.001), higher HDL cholesterol (p<0.001), higher LDL cholesterol (p<0.001), higher fasting blood glucose (p<0.001), higher 2-hour postprandial blood glucose (p<0.001) and higher HbA1C levels (p<0.001).

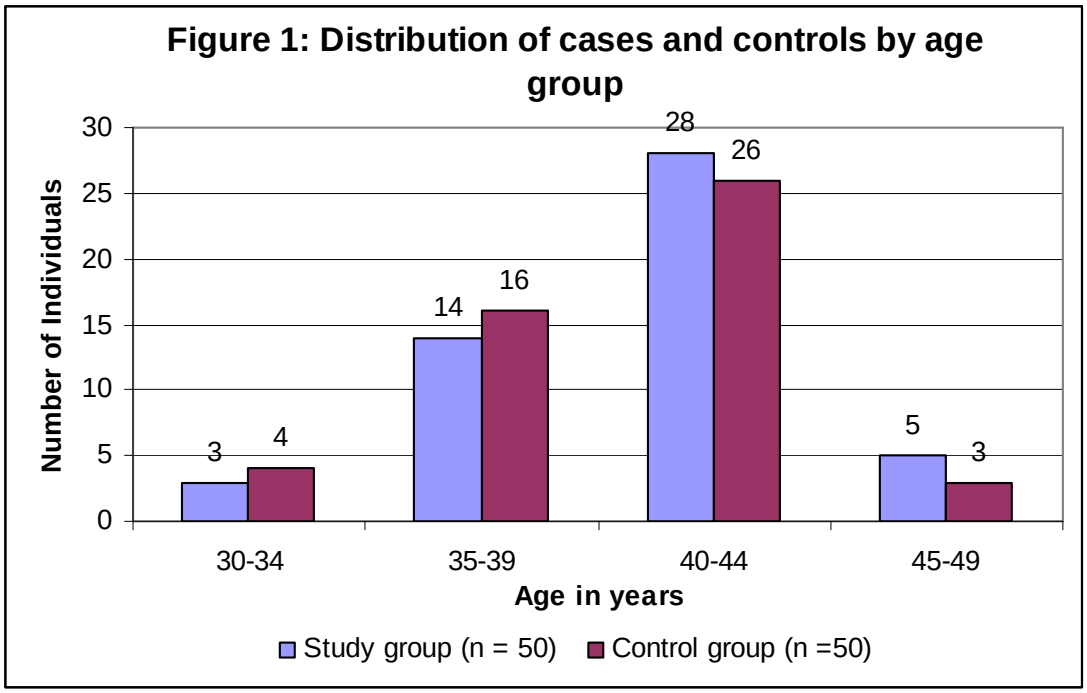


Figure 1: Distribution of cases and controls by age group
Number of Individuals
30
25
20
15
10
5
0
3
4
14
16
28
26
5
3
30-34
35-39
40-44
45-49
Age in years
Study group (n = 50)
Control group (n =50)

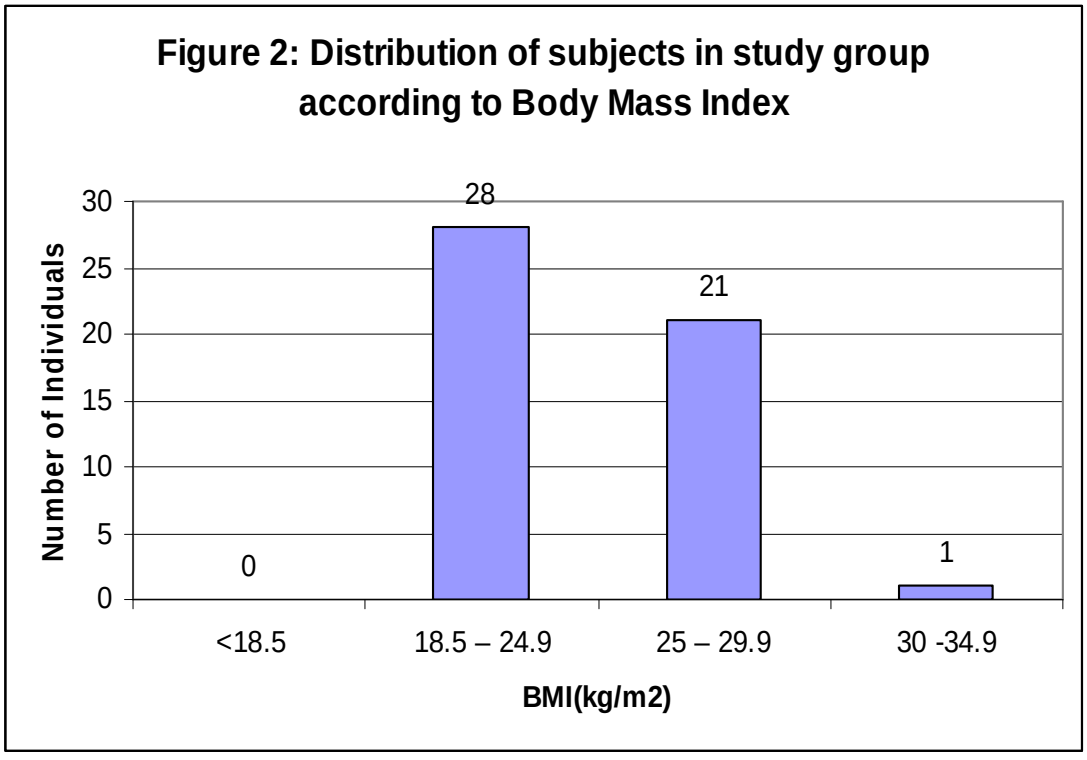


Figure 2: Distribution of subjects in study group according to Body Mass Index
Number of Individuals
30
25
20
15
10
5
0
0
28
21
1
<18.5
18.5 – 24.9
25 – 29.9
30 -34.9
BMI(kg/m2)

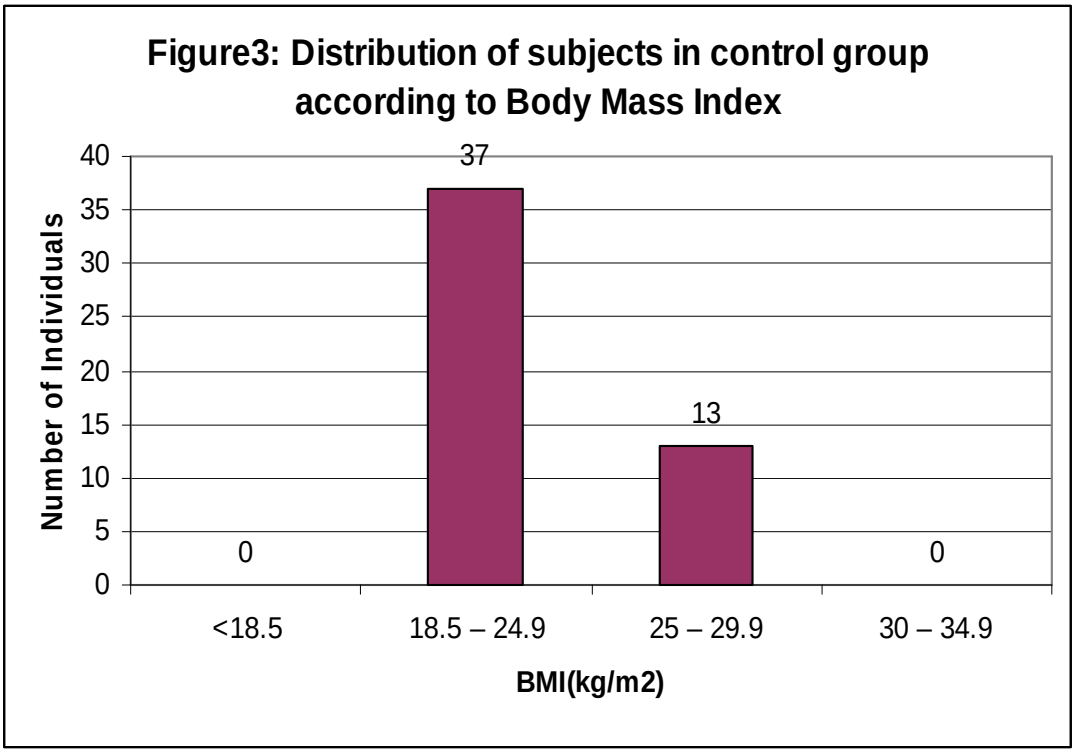


Figure3: Distribution of subjects in control group according to Body Mass Index
Number of Individuals
40
35
30
25
20
15
10
5
0
37
13
0
0
<18.5
18.5 – 24.9
25 – 29.9
30 – 34.9
BMI(kg/m2)

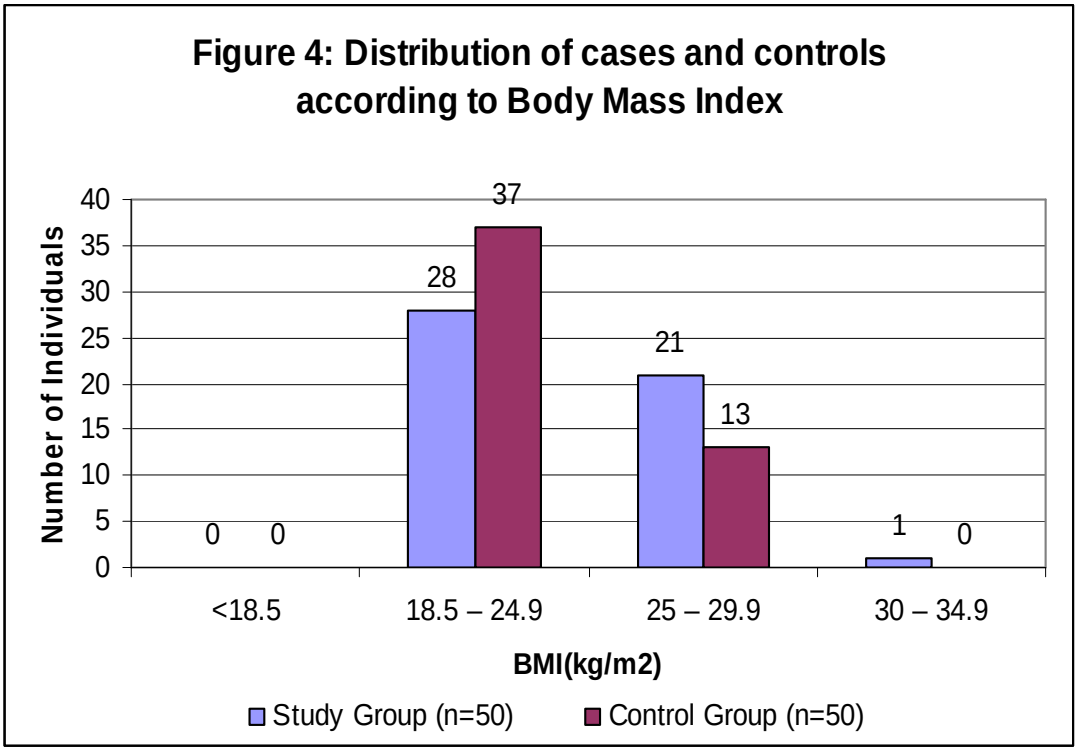


Figure 4: Distribution of cases and controls according to Body Mass Index
Number of Individuals
40
35
30
25
20
15
10
5
0
37
28
21
13
0
0
1
0
<18.5
18.5 – 24.9
25 – 29.9
30 – 34.9
BMI(kg/m2)
Study Group (n=50)
Control Group (n=50)

Fiqure 5: Hypertension in the study group and control group

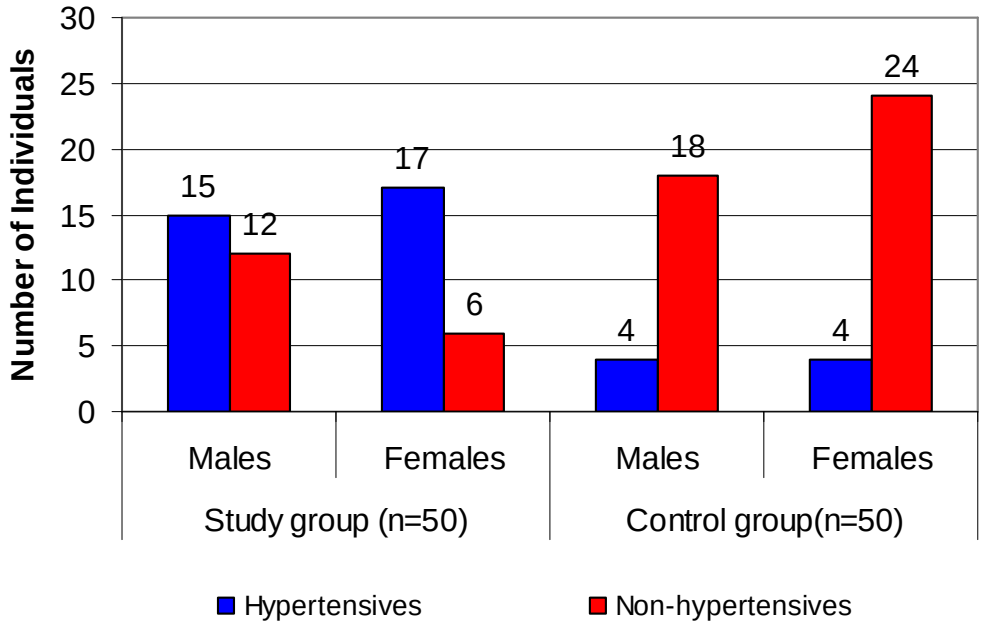
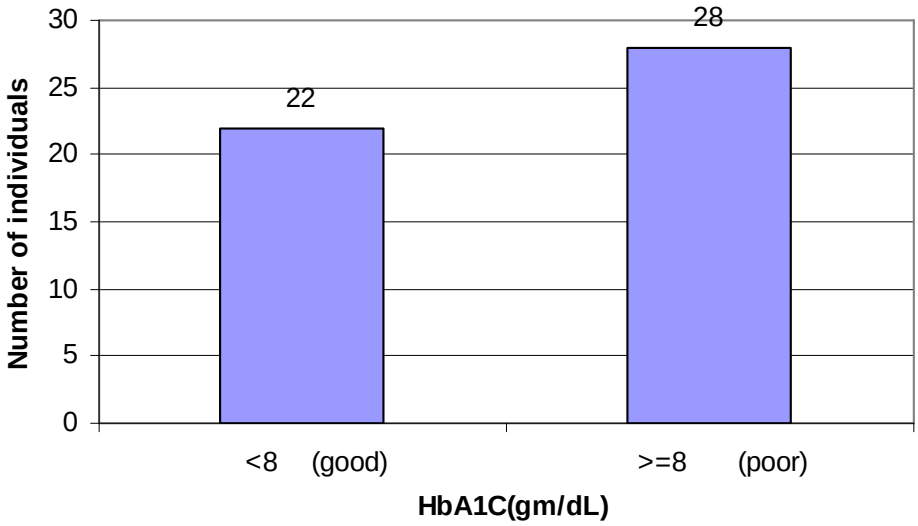


Figure 6- Distribution of cases according to glycemic control



DISCUSSION

Diabetes is now considered to be a proinflammatory and procoagulant state. Diabetes and insulin resistance are associated with a procoagulant milieu that may promote atherothrombosis by facilitating the expansion of nascent thrombi generated by plaque disruption[156]. The elevated levels of coagulation factors, persistently activated thrombogenic pathways and impaired fibrinolysis in patients with diabetes predisposes them to arterial thrombosis[157,158]. Fibrinogen level has emerged as an important and independent predictor of cardiovascular disease and coronary events in a number of prospective studies including the Gothenburg, Northwick Park Heart Study[153] and the Framingham Heart Study[149].

Diabetes is also a proinflammatory state and low grade chronic inflammation ('microinflammation') is a key factor in the genesis and rupture of atheromatous plaque[127]. Highly sensitive C-reactive protein, a marker of inflammation, has also emerged as an important, independent predictor of cardiovascular risk in various studies[159,160].

In this study an attempt was made to compare the risk of coagulation marker fibrinogen and the inflammatory marker, hsCRP in type 2 diabetics and matched controls.

The study and control population were comparable with respect to age (41.10 ± 4.00 in study group vs 40.04 ± 4.28 in control group; p 0.204). Diabetic subjects have higher Body Mass Index (BMI) in this study. This was further confirmed by the study done by Ni Mhurchu et al. (2006)[161]. Cosin Aguilar et al. (2007) from his study state showed higher prevalence of diabetes in the obese patients[162]. NHANES (2005) report and Eric and John (2006) indicates that most adults with diagnosed diabetes were overweight or obese, prevalence of overweight or obesity was 85.2 % and the prevalence of obesity was 54.8% [163,164].

Systolic and diastolic blood pressure was higher in diabetic subjects. David and Paul[165] (2004) showed that the blood pressure and blood pressure progression were strong and independent predictors of incident type 2 diabetes among initially healthy women. The NHANES (1988-1994) disclosed that 71% of diabetic individuals were found to have hypertension (Geiss, et al. 2002)[166].

The study group also had higher levels of serum total cholesterol, triglycerides, LDL cholesterol and lower levels of HDL cholesterol. Diabetic dyslipdemia (Erkelens)[92] is well known in various studies including the Heart Protection Study (HPS)[94], Anglo-Scandinavian Cardiac Outcomes Trial-Lipid-Lowering Arm (ASCOT-LLA)[96], the Collaborative Atorvastatin Diabetes Study (CARDS)[97], the Lescol Intervention

Prevention Study (LIPS)[99], and the Cholesterol and Recurrent Events (CARE) study.

In our study, the fasting blood glucose, 2-hour postprandial blood glucose and Hb1Ac was higher in the diabetic subjects than normal subjects. The present study showed a significant increase of hsCRP in subjects with type 2 diabetes (3.4 ± 1.16 in diabetics vs 2.35 ± 0.65 in controls). This is in accordance with several studies including a study by Yuan G et al. in 2006 which showed that in comparison with normal control (healthy people) serum hsCRP was significantly increased in impaired glucose tolerance (IGT) and type-2 diabetes mellitus group ($P<0.001$), although there was no significance between the two groups[167].The Pickup's group(1997)[113], the Tan's group (2002)[114], Ford and Co-workers in the NHANES III(2003)[115] ,all demonstrated a higher level of hsCRP in diabetics than in those with normal fasting glucose. Also Graham T.E et al. in 2006 showed that subjects with type 2 diabetes had higher baseline levels of C-reactive protein, interleukin-6 and free fatty acids than those with impaired glucose tolerance[168]. In a study in Bangladesh in 2007 by Rosy N, showed that hsCRP was significantly higher in Gestational diabetes mellitus (GDM) group (9.56 mg/L) than in controls (normal pregnant group, 2.19 mg/L)[169].

There are only few studies of hs-CRP in Asian Indians, a very high-risk group for diabetes. (Mohan et al. 2001; Mohan et al. 2003; Wild et al. 2004)[170-172]. There are studies by western authors which shows that Asian Indians has higher levels of hsCRP. In a study by Chamber et al. in the year 2001, the mean CRP concentration was observed to be 17% higher in Indian Asian than with European White[173].

In our study, hsCRP did not have statistically significant correlation with age in the study group, where as it did show a significant positive correlation in the control group. The elevation in hsCRP with increasing age is well demonstrated in other studies by Rieko Hayaishi-Okano et al. (2002)[174], Safiullah Amanullah et al (2010)[175], Rudrajit Paul et al (2011)[176] and various other studies. This variation in our study can probably be attributed to the small sample size.

In our study, serum hsCRP levels positively correlated with anthropometric variables such as Body Mass Index (BMI), systolic and diastolic blood pressure, serum total cholesterol, serum triglycerides, LDL cholesterol and inversely with HDL cholesterol levels which is accordance with earlier studies (Francisco et al. 2005[177]; Li CZ et al. 2004[178]; Taniguchi et al. 2002[179]; Earl and Wayene, 2004[180]; Wu et al.2006[181], Festa et al.2000[120]). Pick up et al. in 1997 showed positive correlation between hyper triglyceridemia and diabetes mellitus and CRP levels[113]. Other

studies have shown that CRP levels are significantly associated with obesity (Visser et al. 2000[182]; Pradhan et al. 2001[183]).

The correlation of hsCRP with fasting plasma glucose and HbA1c observed in our study in diabetics is similar to the previous studies (Li CZ, et al. 2004[178]; Pradhan et al. 2001[183]). Aronson et al. in 2004 in a population based study showed hsCRP to be independently associated with fasting plasma glucose[184]. In our study serum hs CRP levels showed positive correlation with glycemic control (HbA1C). The relationship of hsCRP with glycemic control and its influence on inflammation was shown in a prospective study by Rodriguez and Guerrero, in 1993 on type 2 diabetic subjects which suggested a decrease in hsCRP levels with a decrease in HbA1C[185].

In our study we characterized diabetics as high risk using an hsCRP cut-off value >3.0 mgs /L .We found that the high risk subjects with hsCRP>3.0 mgs/L had higher body mass index, systolic blood pressure, diastolic blood pressure, serum total cholesterol, triglyceride, LDL cholesterol, fasting plasma glucose, 2-hour postprandial plasma glucose and HbA1c levels and lower HDL cholesterol than the subjects with normal hs-CRP (hs-CRP < 3.0 mgs/L). This was in accordance with studies done by Safiullah Amanullah et al. in 2010[175] and Festa et al in 2000[120] .

In our study, plasma fibrinogen levels is significantly elevated in diabetics as compared to controls. This was in accordance with observations of Ceriello A et al. (1994)[186] and Jain A et al. (1999)[187], Wilson PW et al. (2003)[147] and Dunn EJ.et al.(2004)[148].

A statistically significant positive correlation was noted in our study between fibrinogen level and body mass index, comparable to the observation of Krobot et al. in his study[189].In our study, we did not observe significant positive correlation between plasma fibrinogen level and age in the study group whereas age showed significant correlation with plasma fibrinogen in the control group. Krobot et al. in his study showed the positive correlation between age and plasma fibrinogen level[189]. This variation in our study can probable be attributed to the small sample size.

Our study also showed a significant positive correlation between plasma fibrinogen level and hypertension. This was in accordance with a study by Folson et al .1998[190]. In our study, plasma fibrinogen levels also correlated with serum total cholesterol. Similar results were shown by Martin Halle et al.1996[191]. In our study plasma fibrinogen level also correlated with higher levels of triglyceride, LDL cholesterol and lower levels of HDL cholesterol. Similar results were observed in a meta analysis of various prospective studies by The Fibrinogen Studies Collaboration in the year 2007[192].

In our study, a positive correlation was found between plasma fibrinogen and HbA1C level in diabetics. Higher levels of fibrinogen was observed in those with poorer glycemic control. This was in accordance with results from a study by Elkhawnd et al[156]. Kannel B et al, in his study showed that there was an increase in fibrinogen values throughout the range of blood sugar levels in diabetics[149]. Graziella Bruno et al. studied the association of serum fibrinogen level with glycemic control in 1525 patients and found that noninsulin dependent diabetic patients had a high prevalence of hyperfibrinogenemia and that fibrinogen level was independently associated with HbA1c value[150]. In another study, Patnaik B et al. in 1998, had observed that hyperfibrinogenemia can be controlled with adequate control of blood sugar[37].

In our study we found a significant positive correlation between hsCRP and plasma fibrinogen levels in both diabetics and controls. Rudrajit Paul et al.2011[176], Takebayashi Kohzo et al 2006[193], The Fibrinogen studies Collaboration 2007[192] and various other studies also showed significant positive correlation between hs CRP and fibrinogen levels.

However, there is a need to validate the data on larger sample size and in diabetic subjects with cardiovascular disease.

LIMITATIONS OF THE STUDY

1. We would like to acknowledge that a relatively small number of patients were evaluated and that the number of subjects required to detect a significant difference in the predictive power of the hsCRP and plasma fibrinogen has not been prospectively determined.

2. The study is a hospital based study and may not be representative of the general population.

3. In this study hsCRP and plasma fibrinogen levels were estimated only once. Ideally serial measurements should have been done, as both are acute phase reactants.

4. Confounding effects of obesity, hypertension and dyslipdemia have not been taken into account.

CONCLUSIONS AND SUMMARY

- The elevated levels of highly sensitive C-reactive protein (hs CRP) points to the existence of a proinflammatory state in diabetes mellitus.
- The role of inflammation is more pronounced in patients with uncontrolled diabetes when compared to those with glycemic control.
- The elevated levels of plasma fibrinogen points to the existence of a procoagulant state in diabetics.
- Hemostatic alterations are more pronounced in uncontrolled diabetics when compared to better controlled diabetics.
- Body mass index, systolic blood pressure, diastolic blood pressure, levels of serum total cholesterol, triglycerides, LDL cholesterol and HDL cholesterol correlate independently with both hsCRP and plasma fibrinogen levels.
- The levels of hsCRP and plasma fibrinogen correlated with each other.
- Both elevated hsCRP levels and hyperfibrinogenemia may precede clinically demonstrable vascular complications.

- Hence, assessment of hsCRP and plasma fibrinogen levels may help in the evaluation and management of diabetes mellitus, especially to identify individuals with high cardiovascular risk among those without clinically demonstrable vascular complications. This helps to educate them regarding lifestyle modifications and pharmacotherapy of traditional risk factors and the need for good glycemic control with a greater emphasis, in order to prevent cardiovascular complications in the future. Also regular follow up of these high risk patients can lead to early detection of cardiovascular events in the future , thereby reducing economic burden and improving the quality of life.

PROFORMA

A COMPARATIVE ANALYSIS OF HIGH-SENSITIVITY C-REACTIVE PROTEIN (hs-CRP) AND FIBRINOGEN LEVELS IN TYPE 2 DIABETICS AND MATCHED CONTROLS.

DEPARTMENT OF MEDICINE

STANLEY MEDICAL COLLEGE & HOSPITAL

Name :	Age:	Gender :
Occupation :	IP No / OP No :	
Address / Contact No.	DOA: DOD:	

CHIEF COMPLAINTS :

Polyuria	:	+/-	Polydypsia	+/
Polyphagia	:	+/-	Nocturia	+/
Weight loss	:	+/-	Tingling Numbness	+/
Vision	:	+/-	Burning Micturition	+/
Swelling of the feet	:	+/-	Puffiness of face	+/
Skin lesion	:	+/-	Dyspnoea	+/
Claudication	:	+/-	TIA -	+/
Chest Pain (Anginal)	:	+/-	Any Other	
Age at diagnosis	:		Duration	

Prior admissions to hospital for the same:

Treatment	:	Diet + OHA Insulin
Control	:	Good/Fair/Uncontrolled

Other illness (if any) & medications :

Family History of	:		
DM		HTN	:
HD		Any Other	:

Personal History

EXAMINATION :

Build	:		Height	:
Weight	:		BMI	:
Puffiness of face	:	+/-	WC	:
Pulse Rate	:			

PERIPHERAL PULSES :

		RIGHT		LEFT
Carotid	:	+/-	:	+/-
Temporal artery	:	+/-	:	+/-
Brachial	:	+/-	:	+/-
Radial	:	+/-	:	+/-
Femoral	:	+/-	:	+/-
Popliteal	:	+/-	:	+/-
Post. Tibial	:	+/-	:	+/-
Dorsalis pedis	:	+/-	:	+/-

SKIN CHANGES :

Shiny Skin :

Loss of Hair :

Ulcers :

Gangrene

Blood pressure : Supine :

3 Min. standing

ABPI :

Optic fundus

CENTRAL NERVOUS SYSTEM

Other Cranial Nerves :

Peripheral Neuropathy - Ankle Jerk :

Pinprick Sensation :

Vibration Sense :

CARDIOVASCULAR SYSTEM

Heart Sounds : Evidence of Cardiac failure : +/-

RESPIRATORY SYSTEM

PER ABDOMEN

INVESTIGATIONS

CBC - Hb :

TC :

DC :

ESR :

Platelet Count:

BT

CT

RFT - Urea
- Creatinine

Serum Electrolytes - Sodium
- Potassium
- Chloride
- Bicarbonate

FBS

PPBS

HbA_1C

Plasma fibrinogen

hs-CRP

Fasting lipid profile
-Total Cholesterol
-Triglycerides
-HDL
-LDL
-VLDL

LFT

URINE
- Albumin
- Sugar
- Microscopy
- Ketones

TFT

ECG

Chest X – ray

ECHO

MASTER CHART - STUDY GROUP

	NAME	AGE	SEX	Ht(cm)	Wt(kg)	BMI	SBP	DBP	T CH	TG	HDL	LDL	FBS	PPBS	HbA1c	fibrin	hsCRP
1	SELVAKUMAR	32	M	160	63	24.6	124	76	205	170	37	134	136	226	7.3	355	3.1
2	NATARAJAN	34	M	170	71	24.56	126	76	220	197	39	141	134	240	8.1	405	3.6
3	ANTONY	38	M	162	62	23.62	118	70	199	149	49	120	159	281	8.2	410	3.7
4	PUSHPARAJ	36	M	162	63	24	122	74	166	130	57	83	124	198	6.6	280	1
5	PRAMOD	36	M	165	66	24.24	120	76	210	167	37	139	120	198	7.3	352	3.1
6	PRABHAKARAN	39	M	167	60	21.51	124	72	155	133	53	75	114	176	6.6	281	1
7	SELVARAJ	38	M	167	68	24.38	132	82	196	189	36	122	138	230	8.4	421	3.9
8	RAHMAN	37	M	157	60	24.34	140	86	230	190	36	156	150	240	8.6	431	4.2
9	ANKAAIAH	36	M	158	59	23.63	110	70	188	155	59	91	129	198	8.1	390	3.6
10	PANEERSELVAM	39	M	152	56	24.24	122	76	203	163	39	131	125	180	7.3	357	3.1
11	VENKETESH	39	M	161	63	24.3	134	86	201	185	35	129	152	230	8.4	375	3.9
12	MANIKANDAN	44	M	157	61	24.75	136	90	209	177	36	137	148	250	8.2	371	3.7
13	MANIMARAN	43	M	158	61	24.43	136	82	211	189	35	138	132	244	8.7	437	4.3
14	NOOR AHMED	40	M	160	61	23.82	138	84	207	185	35	135	152	249	8.6	433	4.2
15	SELVARAJ	42	M	160	63	22.58	118	74	170	136	56	88	117	180	6.7	290	1.1
16	DEVA JOE	41	M	165	65	23.88	126	74	180	150	56	94	133	231	7.3	352	3.1
17	MEGHANATHAN	44	M	155	53	22.06	114	70	170	134	55	88	106	166	6.6	283	1

	NAME	AGE	SEX	Ht(cm)	Wt(kg)	BMI	SBP	DBP	T CH	TG	HDL	LDL	FBS	PPBS	HbA1c	fibrin	hsCRP
18	MURUGANADHAN	44	M	154	58	24.45	144	82	224	176	37	152	156	197	8.3	416	3.8
19	KUMARAN	43	M	156	56	23.01	120	70	168	146	52	88	102	158	6.7	290	1.2
20	THANKARAJ	42	M	162	65	24.77	140	84	240	180	35	169	147	205	8.5	428	4
21	VIJAYARAGHAVAN	42	M	172	73	24.68	150	80	220	188	35	147	136	254	8.8	444	4.4
22	BEER MOHAMMED	41	M	160	65	25.39	136	92	280	260	30	198	158	260	9	455	4.6
23	KARTHIK	44	M	157	63	25.56	148	86	243	270	34	155	133	206	7.7	371	3.3
24	PANCHALINGAM	40	M	162	70	26.67	144	92	254	189	31	185	145	241	8.3	417	3.8
25	PAUL RAJ	43	M	167	75	26.89	138	90	252	199	30	182	131	223	7.7	373	3.3
26	ANTONY RAJ	48	M	165	70	25.71	160	86	222	189	32	152	155	250	9.1	457	4.7
27	PANCHATJARAM	47	M	163	68	25.59	146	86	214	192	28	147	162	261	8.9	448	4.5
28	PUSHPA	32	F	155	65	27.05	124	72	232	180	32	170	149	249	9.2	463	4.8
29	LATHA	36	F	152	55	23.81	112	70	164	146	54	81	98	170	6.6	276	1
30	AZEERA BEGAM	36	F	157	60	24.34	110	70	212	182	40	135	122	180	7.1	353	3.1
31	GUNASUNDARI	39	F	160	68	26.56	132	90	242	210	30	170	128	241	7.5	360	3.2
32	AMINA	38	F	158	63	25.24	150	90	249	200	30	183	134	228	7.5	363	3.2
33	LOGANAYAKI	38	F	165	70	25.71	142	88	237	191	34	165	161	251	9.2	461	4.8
34	RIYANA	40	F	162	65	24.78	134	86	204	160	36	136	120	194	7.1	352	3
35	VANAJA	41	F	162	63	24	124	72	186	150	59	97	129	214	6.9	315	1.3
36	AMBIKA	41	F	153	54	23.07	122	72	166	142	49	89	107	167	6.7	291	1.1

	NAME	AGE	SEX	Ht(cm)	Wt(kg)	BMI	SBP	DBP	T CH	TG	HDL	LDL	FBS	PPBS	HbA1c	fibrin	hsCRP
37	MAHALAKSHMI	43	F	166	71	25.77	144	82	242	202	36	166	139	234	7.2	361	3
38	SUGUNA	43	F	167	73	26.18	134	90	212	190	34	140	145	251	8.8	442	4.4
39	MANJULA	43	F	155	68	28.3	156	94	250	245	32	169	141	231	8.5	427	4
40	FATHIMA	44	F	158	67	26.84	150	96	260	210	31	187	123	188	7.2	361	3
41	SHANMUGAVALLI	44	F	158	68	27.24	136	84	259	214	33	183	150	257	9	452	4.6
42	MALLIKA	42	F	156	62	25.48	148	92	205	180	34	135	160	242	9.1	457	4.7
43	KALAVATHI	42	F	166	73	26.49	150	88	200	189	30	132	157	231	8.3	413	3.8
44	TAMILSELVI	41	F	160	65	25.39	142	90	220	170	35	151	155	229	8.7	438	4.3
45	YAMUNA	45	F	155	75	30.82	150	100	293	242	34	210	163	252	8.8	443	4.4
46	MALAR	45	F	162	65	24.77	126	76	200	220	35	121	149	246	8.5	423	4
47	BHANU	45	F	158	68	27.24	144	84	212	195	35	138	132	212	7.1	357	3.1
48	MEENAKSHI	48	F	158	60	24.03	144	80	223	179	32	155	142	241	8.6	434	4.2
49	VIJAYALAKSHMI	48	F	157	69	27.99	152	96	251	240	33	169	169	270	9.2	463	4.8
50	KALAICHELVI	49	F	152	60	25.97	160	80	229	196	35	154	136	206	7.5	365	3.3

MASTER CHART - CONTROL GROUP

	NAME	AGE	SEX	Ht	Wt	BMI	SBP	DBP	T CH	TG	HDL	LDL	FBS	PPBS	HbA1c	fib	hsCRP
1	GABRIEL	31	M	162	63	24	120	80	162	130	46	90	82	94	4.1	170	1.1
2	YESUDOSS	35	M	160	60	23.44	130	80	173	128	56	91	78	104	4	182	1
3	PANDIDUARI	35	M	167	65	23.3	132	82	185	142	60	97	73	114	4	194	1.3
4	GANAPATHY	36	M	155	53	22.06	110	70	170	136	56	87	78	110	4.2	250	1.6
5	SARAVANAN	37	M	162	61	23.24	118	70	174	142	58	88	98	120	5.1	210	1.4
6	BABURAJ	38	M	167	65	23.3	120	70	168	108	57	89	80	135	5.3	224	1.5
7	VELLIGOWNDER	38	M	158	62	24.83	130	80	174	130	60	88	93	130	5.4	248	1.6
8	ARUN KUMAR	38	M	170	71	24.57	130	78	180	149	55	95	95	138	5.4	260	1.7
9	SRIDHAR	38	M	174	77	25.43	134	82	220	170	38	148	82	131	4.3	356	3.1
10	RAJKUMAR	39	M	165	70	25.71	130	84	207	189	37	132	81	113	5	351	3.1
11	ELANGO	40	M	162	64	24.39	120	72	170	134	55	88	76	115	5.5	244	1.6
12	RAJA	41	M	166	63	22.86	130	74	163	130	52	85	86	125	5.2	298	2.3
13	BALARAMAN	41	M	155	52	21.64	124	74	170	140	52	90	95	122	5.4	320	2.5
14	MUTHU	42	M	175	68	22.2	126	74	173	138	58	87	85	115	5.1	310	2.4
15	JAADEESHAN	43	M	172	72	24.33	128	80	154	126	47	82	74	110	5.3	320	2.5
16	ARUL RAJ	43	M	169	66	23.1	130	82	176	122	56	96	77	118	5.1	301	2.3

17	ANANDAN	43	M	157	54	21.9	128	80	173	118	52	97	75	98	5.3	267	1.8
18	MANOHARAN	43	M	155	56	23.3	128	80	170	130	59	85	77	102	5	289	2.1
19	SYED BASHA	44	M	171	72	24.62	142	90	214	160	39	143	92	108	5	352	3.1
20	BALAMURUGAN	44	M	170	68	23.53	144	92	175	143	57	89	94	124	4.2	300	2.3
21	AHMED	44	M	158	66	26.44	150	100	240	192	37	164	90	130	5.4	376	3.4
22	CHINNADURAI	48	M	161	67	25.85	148	100	233	177	35	162	85	126	4.6	365	3.3
23	ANBUMALAR	30	F	154	56	23.61	120	70	174	139	59	87	94	114	4.8	310	2.4
24	SINDHU	33	F	158	58	23.23	114	72	166	142	54	84	80	132	5	259	1.7
25	DIVYARANI	33	F	154	60	25.23	112	70	202	159	41	129	82	110	4.8	351	3.1
26	INDUMATHI	35	F	162	65	24.78	116	70	175	134	57	91	75	118	5.2	260	1.7
27	ALANGARAM	35	F	155	52	21.64	118	72	174	118	53	97	78	112	4.9	298	2.3
28	REVATHY	36	F	152	50	21.64	120	74	180	152	58	92	82	100	5.1	271	1.8
29	ABHINAYA	36	F	159	61	24.13	124	76	168	146	52	87	85	120	5	269	1.8
30	SANEETHA	36	F	161	64	24.69	120	70	173	138	54	91	90	125	5.2	310	2.4
31	UMA	37	F	160	69	26.95	134	80	222	175	36	151	93	130	5.4	361	3.2
32	NALINI	39	F	157	63	25.56	130	80	208	171	39	134	94	132	4.9	352	3.1
33	THILAGAVATHY	40	F	155	58	24.14	120	70	168	145	55	84	81	14	4.8	301	2.3
34	SUNDARI	40	F	157	58	23.53	124	72	174	135	52	95	85	130	4.7	299	2.3
35	GEETHA	40	F	165	65	23.88	126	70	172	149	55	87	87	16	4.6	313	2.4

36	SITALAKSHMI	41	F	158	59	23.63	120	72	186	138	59	99	92	114	4.8	325	2.5
37	AROKYA MARY	41	F	158	61	24.44	124	74	177	145	58	90	94	117	4.9	315	2.4
38	CHITHRA	41	F	163	61	22.96	120	70	168	132	48	94	88	105	5	298	2.3
39	ANCY MARY	41	F	152	55	23.8	122	70	170	144	51	90	90	110	5.1	301	2.4
40	GEETHALAKSHMI	41	F	150	55	24.44	120	72	164	146	54	81	85	108	5.4	297	2.3
41	SINDHURI	42	F	150	53	23.56	124	76	166	142	49	89	91	130	5.3	280	2.2
42	INDRANI	42	F	151	55	24.12	124	78	177	135	58	92	90	126	5.2	303	2.3
43	AROKIAAMMAL	43	F	152	60	25.97	144	90	218	181	34	148	86	124	5.2	356	3.1
44	KANAKAMMAL	44	F	155	65	27.06	154	96	229	194	36	154	84	135	4.9	363	3.3
45	AMINA BEGAM	44	F	167	75	26.89	134	84	241	177	37	168	88	19	4.6	374	3.4
46	VANAJAMMAL	44	F	153	63	26.91	152	94	202	200	34	73	76	110	4.7	351	3.1
47	CHINTHAMANI	44	F	164	68	25.28	134	82	223	173	38	150	79	128	4.9	363	3.3
48	ELAKKIAMMAL	47	F	157	59	23.94	130	80	172	148	54	88	93	114	5	278	2.2
49	BHANUMATI	48	F	158	58	23.23	130	80	164	138	46	90	89	132	5.2	298	2.4
50	SHANAZ BEGAM	48	F	156	63	25.89	158	90	219	170	37	143	90	125	4.3	360	3.2

INSTITUTIONAL ETHICAL COMMITTEE, STANLEY MEDICAL COLLEGE, CHENNAI-1

Title of the Work	: A Comparative analysis of High-Sensitivity C-Reactive Protein (hs-CRP) and Fibrinogen levels in type 2 Diabetics and Matched controls
Principal Investigator	: Dr.Teffy Jose PG in MD(GM)
Designation	: PG in MD(GM)
Department	: Department of Medicine Government Stanley Medical College, Chennai-1

The request for an approval from the Institutional Ethical Committee (IEC) was considered on the IEC meeting held on 18.04.2011 at the Modernized Seminar Hall, Stanley Medical College, Chennai-1 at 2PM

The members of the Committee, the secretary and the Chairman are pleased to approve the proposed work mentioned above, submitted by the principal investigator.

The Principal investigator and their team are directed to adhere to the guidelines given below:

1. You should inform the IEC in case of changes in study procedure, site investigator investigation or guide or any other changes.
2. You should not deviate form the area of the work for which you applied for ethical clearance.
3. You should inform the IEC immediately, in case of any adverse events or serious adverse reaction.
4. You should abide to the rules and regulation of the institution(s).
5. You should complete the work within the specified period and if any extension of time is required, you should apply for permission again and do the work.
6. You should submit the summary of the work to the ethical committee on completion of the work.

MEMBER SECRETARY,
IEC, SMC, CHENNAI

ABBREVIATIONS

hsCRP	-	highly sensitive C-Reactive Protein.
Ht	-	Height
Wt	-	Weight
BMI	-	Body Mass Index
SBP	-	Systolic Blood Pressure
DBP	-	Diastolic Blood Pressure
TCH	-	Total cholesterol
TG	-	Triglyceride
HDL	-	High Density Lipoprotein
LDL	-	Low Density Lipoprotein
FBS	-	Fasting blood Glucose
PPBS	-	2-hour postprandial blood glucose
HbA1C	-	Hemoglobin A1C
ADA	-	American Diabetes Association
AHA	-	American Heart Association
WHO	-	World Health Organization
IDF	-	International Diabetes Federation